RECENT ADVANCES IN BONE MARROW TRANSPLANTATION

Robert Peter Gale and Richard Champlin, Organizers

April 13 — April 18, 1986

Plenary Sessions

Poster Sessions

Late Additions

Aplastic Anemia

P0 TREATMENT OF APLASTIC ANEMIA, Richard Champlin, Department of Medicine, Division Hematology/Oncology, UCLA Center for Health Sciences, Los Angeles, CA 90024

Two forms of therapy have been established as beneficial for aplastic anemia, bone marrow transplantation and antithymocyte or antilymphocyte globulin(ATG) . Bone marrow transplantation is a highly effective treatment for young patients with an HLA-identical sibling donor, resulting in 5 year survival in approximately 65% of patients. A number of pretransplant conditioning regimens have been evaluated, most centers have used cyclophosphamide alone or in combination with peripheral blood buffy coat cells. Other centers have utilized cyclophosphamide in combination with either total body irradiation or total lymphoid irradiation. The programs involving irradiation have had a lower incidence of graft rejection but overall survival is similar with each approach. The major complications of BMT include graft rejection, acute and chronic graft-versus-host disease, interstitial pneumonitis and other opportunistic infections. A preliminary analysis of the International Bone Marrow Transplant Registry indicate that the major factors associated with survival include age, with poorer results in older patients, and the development of either graft failure or graft-versus-host disease. Patients receiving transplants from sex-matched donors appear to have superior survival than those receiving sex-mismatched transplants. Survival has been similar in patients receiving post-transplant therapy with either cyclosporine or methotrexate.

Treatment with antithymocyte globulin results in hematologic improvement in 30-70% of patients with severe aplastic anemia. This has been shown to be superior to supportive care alone or treatment with androgens in controlled trials. Results have varied considerably from center to center and between different preparations of ATG; it is currently uncertain whether any particular preparation is superior. The mechanism of action of ATG is uncertain; it may act by immune suppression, altering the regulation of hematopoiesis, by directly inducing proliferation or differentiation of hematopoietic stem cells, or abrogating a population of lymphoid or nonlymphoid inhibitory cells. Responders usually require 2-3 months before any change in peripheral blood counts can be appreciated and these patients continue to have persistent abnormalities of hematopoiesis including macrocytosis, hematopoietic dysplasia and a reduced number of progenitor cells in the bone marrow. Approximately 15% of responders will subsequently develop recurrent aplasia. Combinations of ATG with androgens or with high dose corticosteroids have not substantially improved results achieved with ATG alone. The only clinical factor reported to affect prognosis is the interval from diagnosis to treatment; recently diagnosed patients have a greater chance to respond and patients with intervals >1 year rarely benefit. Age is not a prognostic factor making ATG treatment a preferable approach to older patients even if an HLA-identical donor is available.

P1 ROLE OF T CELLS IN ENGRAFTMENT: EXPERIMENTAL MODELS, CLINICAL TRIALS, Rainer Storb, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

Marrow grafts between DLA-identical littermates following 920 rad body irradiation (TBI) have generally resulted in successful engraftment. However, resistance defined as failure of sustained engraftment has been the rule in DLA-nonidentical littermate and unrelated donor-recipient pairs. The antigens mediating resistance seem coded for by loci close to, but not identical with DLA-A, -B, and -D. Resistance to DLA-nonidentical marrow grafts can be abrogated by very high doses of TBI (1800 rad) or by the addition of viable peripheral blood leukocytes to the marrow inoculum. Failure of enhancement of engraftment by in vitro irradiated leukocytes indicates that cells must be capable of replication to be effective. Addition of thoracic duct lymphocytes instead of blood leukocytes to the marrow inoculum also abrogated resistance to marrow grafts, ruling out the possibility that enhanced engraftment was merely due to infusion of increased numbers of pluripotent stem cells present in blood but not in thoracic duct lymphs. The nature and mechanisms of action of the mononuclear cells from blood and thoracic duct lymph abrogating resistance are currently unknown. They may enhance engraftment by interaction with grafted stem cells or alternatively, abrogate resistance by reacting with residual host immune cells through a graft-versus-host reaction. Resistance to marrow graft is not mediated by classical T cells since treatment of recipients by cyclosporine or antithymocyte serum with/without or procarbazine failed to abrogate it. Treatment with macrophage inhibitors such as silica was partially successful as was treatment of recipients with anti-Ia antibodies with or without postgrafting methotrexate, pointing to a rapidly replicating relatively radioresistant non-T cell.

The existence of resistance in man has only been conjectural. However, as the use of less well matched human marrow donors is being explored, failure of sustained engraftment has been noted, in particular in recipients of T-cell depleted marrow grafts. Whether these failures can be avoided by the addition of certain subsets of lymphocytes to the marrow inoculum is being investigated. Donor buffy coat cells added to the marrow inoculum have been used in multiply transfused patients with aplastic anemia conditioned by cyclophosphamide. This has been accompanied by a decrease in the rate of graft failure from 35% to now 4% with an increase in survival from 45% to close to 80%. A disadvantage of buffy coat cell infusion has been an increased incidence of de novo chronic graft-versus-host disease. Approaches to avoid this problem have included postgrafting immunosuppression with a combination of methotrexate/cyclosporine and, in the future, may involve the infusion of certain subsets of peripheral lymphocytes to enhance engraftment while simultaneously avoiding graft-versus-host disease.

P2 PATHOGENIC MECHANISMS IN APLASTIC ANEMIA, Neal S. Young, Cell Biology Section, Clinical Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892.

The analysis of the pathophysiology of bone marrow failure has become more complex and also more interesting since the realization that an empty bone marrow was not equivalent to a simple deficiency of stem cells. This talk will focus on four aspects of the pathogenesis in aplastic anemia: 1) the immune system as suppressor of hematopoiesis; 2) involvement of viruses; 3) stem cell depletion in PNH; 4) the role of chemicals and drugs. 1) That some cases of aplastic anemia were immunologically mediated was suggested by clinical observations, especially recovery of autologous marrow function in patients conditioned with anti-lymphocyte sera and the failure of marrow engraftment with simple infusion in many syngeneic twins; co-culture experiments appeared to support an inhibitory role for lymphocytes. -interferon is the mediator of hematopoietic suppression in some in vitro systems; interferon production also appears abnormal in patients with bone marrow failure, whose lymphocytes often show abnormally high and dysregulated interferon production. Interferon is detectable in the sera of about 30% of these patients. Interferon in these patients is produced by an activated T suppressor cell, with phenotype Leu 2^+, HLA-DR^+, Tac^+; the proportion of these cells consistently falls with ATG therapy. 2) These lymphocyte and lymphokine abnormalities are similar to those present in human retroviral disease (ATL, AIDS) and raise the possibility that human aplasia may also be a viral disease. The recently discovered B19 parvovirus specifically infects and replicates within human erythroid progenitor cells. The clinical manifestation of B19 infection in normal individuals as fifth disease is dermatologic and rheumatologic, but in the host with underlying hemolysis, B19 causes transient aplastic crisis. However, B19 parvovirus has not been linked to more permanent forms of bone marrow failure, nor is there direct evidence of a retroviral etiology. 3) In paroxysmal nocturnal hemoglobinuria, bone marrow failure may be the result of an intrinsic stem cell abnormality. In vitro studies using specific disease markers have shown that the PNH characteristic develops during erythropoiesis, the majority of progenitors give rise to PNH progeny, and the rate of cell cycling in PNH bone marrow of primitive erythroid progenitors is very high. In combination with clinical data, these data suggest stem cell depletion as a mechanism of bone marrow failure in PNH. 4) Although chemical and drug exposures are probably linked to the majority of cases of bone marrow failure worldwide, least is known of their pathogenic mechanism. Hypothesizing from epidemiologic information and very limited in vitro experiments, it seems likely that "idiosyncratic" disease results from chemical interaction with the immune system or in combination with viral infections, leading to perturbation of the usually quiescent primitive stem cell compartment and bone marrow failure.

Leukemia I

P3 BONE MARROW TRANSPLANTATION IN ACUTE MYELOGENOUS LEUKEMIA, Robert Peter Gale, Department of Medicine, Division of Hematology & Oncology, University of California, Los Angeles, School of Medicine, Los Angeles, CA 90024

Recently bone marrow transplantation from an HLA-identical sibling has been used to treat patients with AML. Favorable results have been achieved. The objective of this presentation is to review current results of transplantation and to compare these with results achievable with chemotherapy.

Transplants in advanced AML achieve 3-5 year leukemia relapse-free survival (LFS) in 10-20% of recipients. This compares favorably with 5% LFS achieved with chemotherapy. Transplants in 2^{nd} remission achieve 20-40% LFS; this is significantly superior to chemotherapy, 5%. Results of transplants in 1^{st} remission are more difficult to critically evaluate. Results of randomized trials are contradictory but clearly indicate that transplantation reduces the liklihood of leukemia relapse significantly; from 70-80% to 20-40%. Survival data suggest no adverse outcome of transplantation and a possible benefit in individuals 15-20 years. Attempts to identify specific high-risk groups who might benefit from transplantation in 1^{st} remission are complex. First, many of these risk factors are contoversial. Second, some risk factors that predict an unfavorable outcome withchemotherapy are likewise operative in the context of transplantation.

In evolving a treatment strategy for AML it is necessary to consider not only the issue of chemotherapy versus transplantation, but also the question of chemotherapy combined with transplantation. In this context it is reasonable to consider the option of initial chemotherapy follow by transplant at relapse. This approach is supported by data suggesting similar outcomes in untreated versus treated 1^{st} relapse.

Other transplant options have been considered in individuals lacking an HLA-identical sib donor. Transplants from only partially mismatched related donors appear to produce similar results in AML patients in remission. This is not true in patients in relapse or with less well matched donors. The latter applies to HLA-haplotype mismatched donors with or without T_{st} cell depletion in vitro. Clearly these approaches cannot be recommended for individuals in 1^{st} remission. Autotransplants have also been investigated. Preliminary encouraging results have been reported in 2^{nd} remission. Whether in vitro treatment of these bone marrows is beneficial is unknown but is likely. Autotransplants in 1^{st} remission have not been performed in a clinical context that permits a critical analysis of whether any benefit has been derived

In summary, transplantation is clearly a useful therapeutic modality in AML but the precise timing to achieve optimal results remains controversial. Furthermore, there is consid=

able difficulty in accurately prospectively identifying those individuals most likely to benefit from transplantation in 1st remission. Mismatched transplants and autotransplants are less clearly of benefit; more data are required to critically evaluate their role

P4 ALLOGENEIC AND AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR ACUTE LYMPHOBLASTIC LEUKEMIA (ALL), John Kersey, Daniel Weisdorf, Mark Nesbit, William Woods, Tucker LeBien, Philip McGlave, Tae Kim, Alexandra Filipovich, Daniel Vallera, Robert Haake, Bruce Bostrom, David Hurd, William Krivit, Anne Goldman, Norma Ramsay, Bone Marrow Transplantation Program, University of Minnesota, Mpls. Minn. 55455

This review will focus on the experience in Minnesota and other institutions with autologous(auto) and allogeneic(allo) marrow transplantation in patients (pts) with high risk ALL transplanted in first or subsequent remission. In Minnesota, 121 high risk pts were transplanted from 1978-1985. Eligible pts for first remission transplant were $\geq$ 21 years of age or pts 16-20 with high risk features (N=19). Children and adults who had relapsed while receiving primary therapy were eligible and represented 84% of total pts; these pts were in second (N=64), third (N=35) or fourth remission (N=3). Median age of pts was 11.9 (range 3-47). Sequential Minnesota protocols utilized cyclophosphamide (CY) +750cGy TBI (N=15), CY +moderate dose Ara C +VM26 +TBI (N=18), CY +fractionated 1320 cGy TBI + random allocation to $\pm$ maintenance chemotherapy with mtx + 6 mercaptopurine (N=73), and 850cGy TBI +high dose AraC (N=15). Pts with an HLA matched sibling donor received an allo transplant (N=77). Since 1982 pts without an HLA matched donor received auto marrow purged *ex vivo* with appropriate B lineage (N=39) or T lineage (N=5) antibody. Allogeneic pts received *in vivo* graft versus host disease (GVHD) prophylaxis with methotrexate(mtx), or mtx, antithymocyte globulin, and prednisone. Comparison of sequential conditioning regimens showed no significant differences relative to relapse or survival. No significant differences were seen between those receiving or not receiving maintenance chemotherapy in relapse or survival. Comparison of auto and allo grafts demonstrate earlier and more frequent relapses in auto cases ($p<.001$) Relapse rates were not different between auto transplants and allo transplants without GVHD, suggesting that the increased relapses in auto pts were due to refractory disease *in vivo* and lack of graft versus leukemia effects, rather than to reinfused leukemia cells. Causes of morbidity were different in allo and auto pts. Forty four pts are alive and relapse free, 23 at 2 to 6.8 years; no relapses have occurred beyond two years. Auto and allo pts have similar projected disease free survival(DFS) and survival(S) at 2 years: DFS=28% $\pm$ 14% (95% confidence interval)(auto) and 34% $\pm$ 16%((allo)($p=.15$); S=31% $\pm$ 18%(auto) and 36% $\pm$ 16%(allo) ($p=.5$) These results suggest that autologous and allogeneic transplantation may have an equal probability of long term survival in high risk ALL and that continuing efforts will be necessary to reduce the relapse rate in this disease.

P5 GRAFT-VERSUS-LEUKEMIA REACTIONS: EXPERIMENTAL MODELS AND CLINICAL TRIALS
Robert L. Truitt, Ann V. LeFever and Charles C-Y. Shih, Medical College of Wisconsin at Childrens's Hospital, 1700 W. Wisconsin Ave., Milwaukee, WI 53233

Substantial progress has been made in methods to avoid graft-vs-host (GVH) disease by elimination of T-cells in allogeneic bone marrow prior to transplantation. However, T-cells which cause GVH disease may mediate an antitumor effect, and the use of T-depletion techniques in patients with malignant disease could result in an increased relapse rate and loss of any survival benefit gained by avoiding GVH disease. Mitsuyau et al.(1) have reported that leukemia recurrence is higher in patients given T-depleted as compared to untreated bone marrow. Although GVH disease may result in a beneficial graft-vs-leukemia (GVL) effect after allogeneic marrow transplantation(2), limited clinical attempts to enhance the GVL effect of GVH disease have not been successful. Described here are the results of studies using AKR mice with advanced T-cell leukemia/lymphoma as an experimental model to evaluate the GVH and GVL effects of bone marrow transplantation. Previous studies from this laboratory have shown that GVL and GVH reactive cells consist of distinct as well as overlapping cell populations(3). In the studies reported here, a therapeutic GVL effect, along with an increase in survival, was obtained only when the GVH reaction was controlled by manipulation of the number of T-lymphocytes in the allogeneic (SJL/J) marrow inoculum. In the absence of a GVH/GVL reaction, residual leukemia recurred in most mice within 28 days after completion of chemoradiotherapy. Survival and GVL benefits were optimum when 5×10^5 lymph node (LN) cells were added to the allogeneic bone marrow. In the absence of added LN cells or when syngeneic cells were used, significant leukemia relapse was observed; increasing the dose of allogeneic LN cells resulted in significant GVH-associated mortality. Both the GVL and GVH reactions correlated with the level of antihost reactive cytotoxic T-cells in the spleens of transplanted mice. These results support the notion that if T-cells are eliminated prior to marrow transplantation, a loss of GVL effect and consequent increase in tumor relapse may occur. Alternate approaches to impart a controlled GVL effect within the context of allogeneic, T-cell deficient bone marrow transplantation are possible. For example, class I antigen ($Qa\text{-}1^b$) specific CTL clones expanded in vitro can mediate a GVL effect in vivo without affecting hematopoietic reconstitution or GVH mortality when given together with allogeneic bone marrow(4).
(This work was supported by USDHHS grants CA18440, CA26245 and CA39854 from the National Cancer Institute and by the Midwest Athletes Against Childhood Cancer Fund and Milwaukee Regional Cancer Center)

(1) Trans Proc 17:482, 1985 (2) Cancer Treat Repts 68:145, 1984
(3) J Immunol 131:2050, 1983 (4) Transplantation 40:531, 1985

Leukemia II

P6 MARROW TRANSPLANTATION IN PATIENTS WITH HEMATOLOGICAL MALIGNANCY RECEIVING GRAFTS FROM DONORS OTHER THAN HLA IDENTICAL SIBLINGS,

John A. Hansen, Patrick G. Beatty, Reginald A. Clift, Jean Sanders, Keith Sullivan, and E. Donnall Thomas, Fred Hutchinson Cancer Research Center, Puget Sound Blood Center, and Department of Medicine, University of Washington, Seattle, WA 98104

As of September 1, 1985, 220 patients with hematological malignancy have been transplanted in Seattle with marrow from a related donor other than an HLA identical sibling. Sixty-three patients had ALL (31 remission, 32 relapse), 92 patients had ANL (39 remission, 53 relapse), 51 patients had CML (17 chronic phase, 34 acccelerated or blast crisis), and 14 patients had other hematological malignancies. Donors and recipients were haploidentical family members, and thus were genotypically identical for two of their four haplotypes. The degree of disparity between the remaining two haplotypes was variable: 25 patients were phenotypically compatible with their donor for HLA-A,B and D (MLC-defined); 104 were incompatible for 1 locus (A, B, or D); 74 were incompatible for 2 loci; and 17 were incompatible for 3 loci. Patients were assigned to primary treatment protocols appropriate for their particular disease, and all patients received unmodified donor marrow (except when depleted of plasma or red cells because of ABO incompatibility). The first 194 patients (transplanted through April 1985) received standard post-transplant methotrexate.

Since May 1, 1985, all patients have received methotrexate (on days +1, +3, +6, and +11) and cyclosporine (beginning on day -1). Results for the "study" group were compared to external controls consisting of patients with comparable disease transplanted in Seattle during the same period of time. The median time required for peripheral nucleated blood cells to reach 500 and 1,000/mm^3 was comparable in study patients and controls, but a significant number of study patients had persistent granulocytopenia (less than 1,000/mm^3) beyond day 40. There was also a singificant increase in graft rejection in study patients (3.5% vs 0.1%). Rejection correlated with two factors, total dose of TBI (12 Gy vs 15.8 Gy), and the extent of HLA disparity. The incidence of rejection for a 1-locus incompatible graft was 2%, while the incidence of rejection for 2- or 3-loci incompatible grafts was 7%. Overall incidence of AGVHD was significantly greater in study patients compared to controls. Risk of AGVHD was not significantly different for class I (HLA-A or B) versus class II (HLA-D disparity. Patients transplanted in remission from a donor incompatible for one HLA locus (A, B, or D) had a probability of disease-free survival that was not significantly different from controls transplanted in remission.

P7 CHIMERISM FOLLOWING TRANSPLANTATION: IMPLICATIONS FOR GRAFT-FAILURE, GVHD, AND LEUKEMIA RELAPSE, L.D. Petz, P. Yam, R.B. Wallace, M.T. Gallagher, D.R. Branch, A.D. Stock, V. Brown, R. Knowlton, H. Donis-Keller, G. de Lange and K.G. Blume, City of Hope National Medical Center, Duarte, CA, Collaborative Research Institute, Lexington, MA, and Red Cross Blood Transfusion Service, Amsterdam, The Netherlands.

We have previously demonstrated stable mixed hematopoietic chimerism (mixtures of donor and recipient hematopoietic cells) in 9 patients treated with high-dose pre-BMT radiochemotherapy prior to receiving marrow from histocompatible sibling donors for acute leukemia. Mixed chimerism in our initial studies was documented by RBC antigens, chromosomes, immunoglobulin allotypes and granulocyte antigens. The aims of our present study are to determine the incidence of mixed chimerism after various pre-BMT preparatory regimens in order to understand the factors leading to its development; also, to determine the effect of mixed chimerism on the incidence and severity of acute and chronic GVHD, and on relapse after BMT for acute leukemia. Further, we have studied the feasibility of using DNA restriction fragment length polymorphisms (RFLP) to document mixed chimerism, donor engraftment, or endogenous marrow repopulation. Our preliminary results suggest a higher incidence of mixed chimerism with a pre-BMT regimen consisting of 750 or 1000 rad TBI given in a single dose, cyclophosphamide (CYT) 100 mg/kg, and arabinoside-C (Ara-C) 5 mg/kg 2 times compared with a regimen consisting of fractionated TBI to a dose of 1320 rad and CYT or VP-16 (but no Ara-C). Only one of our 9 reported stable mixed chimeras all of whom were transplanted for acute leukemia has had recurrent disease after periods of follow-up from about 3 to 5½ years, and none have had severe GVHD (grade III or IV). Studies of DNA RFLPs have been carried out in 3 previously documented stable mixed chimeras, 5 previously transplanted patients and donors, and in 19 prospectively studied donor/recipient pairs. Our results demonstrate informative RFLP markers in all cases studied. Mixed chimerism can be detected even when donor or recipient DNA is only about 5% of the total and engraftment can be demonstrated with RFLP when other genetic markers are inconclusive, especially in sex-matched BMT. These findings indicate that definitive documentation of complete allogeneic marrow engraftment is important and that mixed chimerism after BMT for acute leukemia does not preclude long term disease-free survival. GVHD in mixed chimeras may be decreased in incidence and in severity and this observation may lead to new insights regarding tolerance.

Acute Graft-vs-Host Disease

P8 PROGNOSTIC FACTORS FOR ACUTE GVHD. Mortimer M. Bortin, M.D., for the Advisory Committee of the International Bone Marrow Transplantation Registry, Milwaukee, WI 53226.

Analyses were performed to identify factors predictive of moderate to severe AGVHD in 1871 patients reported to the IBMTR by 98 transplant teams, worldwide. All 608 patients with AML, 549 with ALL, 367 with CML, and 347 with SAA transplanted between 1978–1984 using HLA-identical sibling donors who survived ≥ 21 days with engraftment were included in the study. The incidence of moderate to severe AGVHD was 44%; actuarial probability 45%; mortality rate 21%; and case fatality rate 48%. AGVHD began in 77% within 30 days and in 95% within 60 days posttransplant. The incidence of 51% in CML was higher ($P<0.003$) than the 39% incidence in SAA. The case fatality rate was higher ($P<0.002$) in SAA (61%) than in leukemia (46%). The risk of AGVHD was lower ($P<0.006$) in younger (39%) than in older patients (46%), and lower ($P<0.003$) in females (40%) than in males (47%). The incidence was highest ($P<0.0001$) when female donors were used for male recipients (53%) vs other sex-match combinations (41%), and the risk in female → male transplants was significantly increased ($P<0.006$) when female donors had been alloimmunized via previous pregnancies or transfusions (64%) vs non-alloimmunized female donors (49%). Use of methotrexate (43%) or cyclosporine (46%) had similar incidences of AGVHD, whereas T-cell depletion of donor marrow pretransplant was associated with a significantly lower ($P<0.006$) rate (31%). The results of this study may help identify patients prospectively who are at increased risk of developing moderate to severe AGVHD.

P9 PROPHYLAXIS AND TREATMENT OF GRAFT-VERSUS-HOST DISEASE (GVHD) WITH DRUGS, H.J. Deeg, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

Studies in experimental animals have shown that GVHD can be prevented or treated with immunosuppressive agents. Based on those studies, methotrexate (MTX), cyclophosphamide (CY), prednisone (PD), antithymocyte globulin (ATG), cyclosporine (CSP) and other agents have been used in clinical marrow transplantation. With MTX or CY, the incidence of acute GVHD at various centers was 40-70% with HLA-identical marrow transplants. Initial studies with CSP suggested that the incidence of GVHD would be lower. Prospective randomized studies, however, show that even with this agent the incidence of acute GVHD may be as high as 50%. Based again on experimental data, a regimen combining MTX and CSP was used clinically. Randomized studies indicate that with such a regimen the incidence of acute GVHD may be as low as 20-30% for patients in all age groups. A reduced incidence of GVHD has also been reported with a regimen combining MTX, ATG and PD. - In patients who, despite prophylaxis, develop acute GVHD, agents such as PD, ATG, and CSP have been used for treatment. PD has been considered standard therapy. Randomized studies indicate that PD and ATG are equivalent with approximately 30% of patients responding. Recent studies suggest that in patients on MTX prophylaxis, CSP for treatment of established GVHD may be superior to PD or ATG with approximately 50% of patients responding. Further improvement was observed with the use of a combination of ATG and CSP with approximately 60-70% of patients responding to this therapy. The optimum regimen for patients on CSP prophylaxis still needs to be defined.

P10 Clinical Trials of T-lymphocyte Depletion in Man

Robert Peter Gale, Department of Medicine, Division of Hematology & Oncology, University of California, Los Angeles, School of Medicine, Los Angeles, CA 90024

Most data indicate that T-lymphocytes are responsible for GvHD in transplants between HLA-identical and HLA-mismatched donors - recipients. Because of this, recent clinical trials here evaluated the efficacy of T-cell depletion in preventing GvHD. Techniques utilized include antisera or monoclonal antibodies to T-cells (with or without complement or immunotoxins) and physical techniques such as depletion of E-rosette forming cells or lectin-assisted fractionation. This study critically reviews transplants using T-cell depletion in patients with hematologic disorders. Outcomes to be investigated include: (1) prevention or modification of GvHD; (2) graft-failure (rejection); (3) leukemia relapse; (4) survival.

Approximately 500 individuals received T-cell depletion bone marrow transplants; >95% had acute or chronic leukemia. These data were compared to results in 2000 recipients of HLA-idential sibling grafts and to 500 HLA-mismatched grafts that were not T-cell depleted.

GvHD was reduced by T-cell depletion in recipients of HLA-identical transplants. Comparable results were obtaind using anti-T antibodies from different CD groups. Complement and ricin-conjugated antibodies were equally effective, but antibody without complement was not. The type of complement was not important nor was the use of post-transplant suppression.

Graft-failure increased from 1% to 10% in T-cell depleted HLA-identical grafts 30% in HLA mismatched transplants. It was not correlated with the type of T-cell depletion, CD group or postulated reactivity with NK cells of the antibody(s), use of pretransplant cytarabine, dose-rate of radiation, or post-transplant immune suppression.

Survival rates (non-actuarial) were 45% in HLA-identical transplants, and 20% in HLA-mismatched transplants. Since high and low-risk patients were studied, it is not possible to determine if this is different from non-T-cell depleted transplants, but there is no major improvement. One controlled study failed to show a benefit in survival. The risk of leukemia relapse was likewise difficult to critically evaluate because both high and low-risk patients were treated and actuarial rates rarely indicated. In several small series and one controlled trial leukemia relapse appeared to be increased. This increased risk of leukemia relapse was observed in AML and CML and with different conditioning regimens.

These data indicate that T-cell depletion decreased GvHD in HLA-identical transplants and probably HLA-mismatched grafts. Graft-failure is clearly increased; leukemia relapse is probably more frequent, but this is uncertain. It is also unclear that survival is improved. Current trials are focusing on either more selective T-cell depletion or on more effective immune suppressive and anti-leukemia regimens. Controlled randomized trials are needed to determine whether these approaches are successful.

P11 IMMUNOTOXINS (IT) IN BONE MARROW TRANSPLANTATION (BMT), Daniel A. Vallera, Fatih M. Uckun, John H. Kersey, Alexandra H. Filipovich, Dorothea E. Myers and Bruce R. Blazar, Bone Marrow Transplantation Team, Department of Therapeutic Radiology, Laboratory Medicine/Pathology, and Pediatrics, University of Minnesota, Minneapolis, MN 55455.

We have investigated the role of IT as a purgative regimen for GVHD prophylaxis in allogeneic BMT. We chose to link intact ricin to anti-CD3, anti-CD5, and anti-CDw18, three different anti-T cell monoclonal antibodies recognizing different determinants. Our decision to use intact ricin IT was based on selectivity, potency and ease of purification. A phase I clinical trial of 17 matched adult transplants in which donor marrow was pretreated with IT has shown that the strategy is safe and effective for the prevention of severe (Grade 3-4) acute GVHD. No severe GVHD or side effects due to engraftment were measured. Patients engrafted sooner than those receiving conventional GVHD and in many cases were discharged earlier. We and other centers have found that T cell depletion results in graft failure/rejection in 10-30% of the transplants. The reason is unknown. Thus, we have employed a murine model of BMT (BALB/c C57BL/6) to investigate a number of factors which might enhance engraftment. We have tested both donor and host contributions. For example, we tested several radiological conditioning regimens. High single dose TBI, TBI plus cyclophosphamide and fractionated TBI were able to overcome graft failure/rejection attributable to T cell depletion. Since donor engraftment and ensuing immunocompetence are of primary importance, we have employed sensitive DNA-typing in addition to our conventional serotyping to evaluate donor/host chimerism. Restriction fragment length polymorphism analysis was reproducibly more sensitive at a level of 1-10%. We are also using our murine nodel to determine which IT or IT combinations will be best for GVHD prevention and engraftment. Although depletion of donor helper T cells was more effective in GVHD prevention than elimination of donor cytotoxic T cells, the elimination of both populations yielded the most effective prophylaxis (76% survival at day 70 post transplant). For those patients lacking compatible donors, we devised and applied an IT protocol in which autologous marrow is purged of residual leukemic T cells. In a phase I trial, seven patients with T cell leukemia/lymphoma received IT-treated marrow. Treatments were with anti-CD5/anti-CDw18 or anti-CD5/anti-CDw18/anti-CD3 depending on marker expression on malignant cells. The procedure was without side effects and all patients engrafted. Two patients continue relapse-free at 8+ and 15+ months post transplant. Experiments are in progress to optimize the current autologous purging protocol based on the expression of T lineage surface markers on clonogenic leukemic blasts. The IT sensitivity of blast progenitors have been measured in a novel culture system. We find that blasts express CD2, CD5, and CD7 markers and are snesitive to IT at the ribosomal level.

Chronic GvHd and Immune Deficiency

P12 INTERSTITIAL PNEUMONITIS FOLLOWING BONE MARROW TRANSPLANTATION (BMT) PATHOLOGICAL STUDY OF 35 CASES.

A JANIN-MERCIER, A DEVERGIE, E VILMER, S VALADE, L BOCCON GIBOD, MF D'AGAY E GLUCKMAN. Unité Fonctionnelle de Moëlle Osseuse (UFGM) Service d'Hématologie du Pr M. BOIRON - HOPITAL SAINT LOUIS 1, av Claude Vellefaux 75475 PARIS Cédex 10 FRANCE.

Lung biopsies of 35 patients with interstitial pneumonitis following BMT have been studied histologically, ultrastructurally and by immunofluorescence. Among infectious diseases, cytomegaloviroses were the more frequently found and were related to severe chronic graft versus host disease in allogenic or mismatched BMT. Hemorragic pulmonary oedema, disseminated intra-vascular coagulation and eosinophilic pneumonitis were observed but in 11 cases, the diffuse interstitial pneumonitis was "idiopathic". 2 patients had concomittant diffuse lung fibrosis, cutaneous sclerosis and Sjögren-like syndrome. The pulmonary and cutaneous scleroses had common features in the type of collagen and in the composition of the infiltrate. Both fibrosis might result from a common pathogenic mechanism related to an immunologic conflict between the lymphocytes of the graft and the cells from the host tissues.

P13 EXPERIMENTAL MODELS OF GVHD. Brian L. Hamilton, Department of Biological Structure, University of Washington, Seattle, WA 98195.

Experimental models of GVHD have been developed in a number of species including nonhuman primates, dogs, and a varity of rodents. Inbred strains of rats and mice have been especially useful in experimental studies of the mechanisms of GVHD in recipients of both MHC incompatible and MHC compatible, but minor histocompatibility antigen (minor HA) incompatible, marrow transplants. Using inbred strains, the clinical appearance and median survival times (MST) of mice with GVHD have been demonstrated to be a complex function of several variables. In MHC incompatible transplants the critical variables include: 1) The MHC haplotype to which the donor immune system responds, 2) The specific subregion of the MHC involved, and 3) The number of donor T cells included in the marrow inoculum. In the case of MHC-compatible transplants (minor antigen GVHD) critical variables include: 1) The number of minor HA by which donor and recipient differ, 2) The specific minor HA alleles to which the donor immune system responds, 3) The MHC type of the donor-recipient combination, 4) The number of donor T cells included in the marrow inoculum, and 5) The antigenic experience of the donor (e.g. specific immunization, infections, etc.).

Attempts to determine the immunologic mechanisms of GVHD have generated apparently contradictory results. Using both negative and positive selection methods, several studies have shown conclusively that mature donor T cells are necessary for the initiation of MHC and minor antigen GVHD. It is less clear whether specific subsets of T cells are either necessary or sufficient to initiate GVHD. In some experimental models the cells that initiate GVHD are Lyt-2^+. In other models the initiator cells are Lyt-2^-, L3T4$^+$ and, in some, both Lyt-2^+ and L3T4$^+$ cells are necessary to initiate GVHD.

The actual effector mechanisms of GVHD are not well defined. Evidence for and against a primary role of Cytolytic T Lymphocytes (CTL) as effectors of GVHD have been presented. Both MHC Class I and MHC Class II restricted CTL have been described in animals with GVHD. Evidence that Natural Killer (NK) cells contribute to the pathogenesis of GVHD has recently begun to emerge. Nonspecific suppressor cells, autoimmune phenomena (both humoral and cellular) and significant immune dysfunction also contribute to GVHD in some models.

GVHD appears to represent a complex immunologic reaction that is initiated by antigen specific T cells and involves several mechanisms at the effector level, including cytotoxic lymphocytes (CTL and NK), delayed type hypersensitivity (DTH) reactions involving a number of lymphokines, and alloantibody. The immune dysregulation that results in autoimmune phenomena and immunodeficiency syndromes may develop in response to the primary immunologic reaction to alloantigens. The specific subpopulations of T cells that initiate GVHD and the dominant effector mechanisms of GVHD appear to be a function of the specific minor HA and MHC type of the strain combinations transplanted.

P14 IS CHRONIC GVHD AN AUTOIMMUNE DISORDER? Robertson Parkman, Yves DeClerck and Sharyn M. Walker, Department of Pediatrics, Division of Research Immunology/Bone Marrow Transplantation, Childrens Hospital of Los Angeles, Los Angeles, CA 90027
Classically graft versus host disease (GVHD) has been assumed to be due to the immunoagression of mature donor T lymphocytes infused at the time of bone marrow transplantation. The donor T lymphocytes expand following exposure to recipient specific histocompatibility antigens and differentiate into effector cells that produce clinical GVHD. Evaluation of a murine model of non-MHC GVHD (LP $\longrightarrow$ C57BL/6) by the analysis of T lymphocyte clones derived from animals with acute (Day 14) and chronic (Day 50) GVHD has revealed the following 1) clones from acute GVHD mice are primarily cytotoxic T lymphocytes with B6 specificity and 2) clones from chronic GVHD mice are always non-cytotoxic with specificity for autologous I-A^b. In addition some I-A^b specific clones are derived from acute GVHD mice. Following stimulation with B6 spleen cells the I-A^b specific clones release a leukokine that stimulates collagen synthesis by fibroblasts. Thus, the increased collagen disposition, that is characteristic of chronic GVHD, may be due to auto-reactive helper T lymphocytes rather than cytotoxic T lymphocytes with recipient antigen specificity. Further the auto-reactive T lymphocytes may directly stimulate B lymphocytes to produce the auto-antibodies that characterize chronic GVHD. Most of the clinical symptomatology of chronic GVHD can be explained by the presence of auto-reactive T lymphocytes.

P15 MECHANISMS OF POST-TRANSPLANT IMMUNE RECONSTITUTION, Andrew Saxon, Ron Mitsuyasu, Ronald Stevens, Richard Champlin, Hajime Kimata and Robert P Gale Departments of Medicine and Microbiology/Immunology, University of California, Los Angeles, CA 90024.

A major obstacle to the successful outcome of bone marrow transplantation is the immunodeficiency seen in recipients who demonstrate a quantitative and qualitative immunodeficiency involving both humoral and cellular immunity. While the number of circulating B cells returns to normal within 2-3 months, absolute levels of various immunoglobulins remain depressed for up to 1 year and specific antibody responses are abnormal well beyond this. Similarly, while total T cell numbers recover within months, there are persistant functional abnormalities such as impaired delayed hypersensitivity responses and an altered distribution of helper (CD4) and suppressor (CD8) phenotype T cells. These abnormalities are felt to reflect the immaturity of the immune cells engrafted, cells that require development within the donor to express an effective immune response.

To test this, we took advantage of the ability to specifically immunize marrow donors pre-transplant and thereby antigen specific activated B cells in the marrow at the time of transplantation. Donors were immunized with tetanus (tet) and diphtheria (dip) toxoids 1 week before bone marrow donation in order to investigate the role of functional subsets of B cells in the reconstitution and expression of humoral immunity in the recipients. Samples of the transferred marrow were tested in vitro for B cells that made specific antibody spontaneously or in response to pokeweed mitogen and T cell help. In 7 of 7 cases, lymphoblastoid B cells that spontaneously produced IgG anti-tet and anti-dip were detected in the donor marrows at the time of transplantation while pokeweed mitogen reactive antigen specific cells were absent in all cases. The 7 recipients demonstrated a 2-90 fold increase in their serum IgG anti-tet and anti-dip toxoids levels. These levels were achieved within 3 weeks of marrow grafting and often as early as 1 week after transplant. Spectrotype analysis of the anti-diphtheria Fragment A antibody by isoelectric focussing in donor-recipient pairs was informative in two cases. This revealed clonotypic identity of the antibody in these cases indicating that we had achieved the adoptive transfer of the donor's specific IgG response to the recipient. Reimmunization of 3 recipients with tet-dip 2 to 5 months after transplant revealed a characteristic secondary immune response with the development of an IgG anti-tet in the absence of an IgM response. Furthermore, spectrotype analysis revealed that the anti-diphtheria Fragment A response was a secondary one that showed the clonotype characteristic of the marrow donor's immune response. This suggests that the transplantation of specific lymphoblastoid B cells was also capable of transferring specific immune memory. These findings suggest that through designed immunization of donors, it will be possible to specifically transfer both adoptive ongoing humoral immunity and B cell immune memory for clinically relevant pathogens.

P16 CHRONIC GRAFT-VERSUS-HOST DISEASE (GVHD) IN MAN, Keith M. Sullivan for the Seattle Marrow Transplant Team. Fred Hutchinson Cancer Research Center, Seattle, WA 98104

Chronic GVHD with associated late infection is observed in 25-40% of long-term survivors of allogeneic marrow transplantation. Older patient age, prior acute GVHD and infusion of viable donor buffy coat cells increase the risk of developing this complication. Prolonged immunodeficiency may result from generation of nonspecific suppressor T cells and predispose to recurrent late infections. Major nonvaricella zoster infections developed after day 100 posttransplant in 10 of 20 patients with untreated chronic GVHD. Late interstitial pneumonia (IP) was observed in 15% of these untreated patients. Among 178 patients treated with prednisone-based regimens for chronic GVHD, IP developed in 8% of those given trimethoprimsulfamethoxazole (TMP-SMX) prophylaxis and 28% of those not given TMP-SMX ($p=0.001$). Two forms of clinical chronic GVHD have been described: patients with limited disease (skin and/or liver only) have a favorable outcome without treatment, while patients with extensive (multiorgan) disease have only a 20% disability-free survival without therapy. Histologic diagnosis is established by skin and oral biopsies. Early diagnosis before clinical deterioration has resulted in apparent improved response to therapy. Results of a recent randomized trial showed early treatment with prednisone (1.0 mg/kg/QOD) and TMP-SMX (1 DS BID) was associated with fewer infections and improved survival compared to patients treated with prednisone, TMP-SMX and azathioprine. The nonleukemic mortality in the two groups was 12% and 37%, respectively. Scleroderma can be prevented, but irreversible obstructive airway disease is an ominous late development in 5-10% of chronic GVHD patients and resembles obliterative bronchiolitis. Patients with "high risk" chronic GVHD are defined as those failing initial therapy or those presenting with <100,000 platelets/mm^3 at diagnosis. Transplant related mortality in these high risk patients was 62% after therapy with prednisone and TMP-SMX. Recently, 45 high risk patients were treated with TMP-SMX prophylaxis and an alternating day regimen of prednisone and cyclopsporine. Although results are preliminary, the Kaplan-Meier estimate of 2-year survival is 76%. Prevention of late IP and infection and tailoring of immunosuppressive treatment to prognostic subgroups of patients with chronic GVHD remain important aspects of the long-term care of allogeneic marrow recipients.

P17 MECHANISMS OF CHRONIC GVHD, Peter J. Tutschka, The Ohio State University Bone Marrow Transplantation Program, Columbus, Ohio 43210.

Chronic graft-versus-host disease (cGVHD) has emerged as a distinct entity in clinical marrow transplantation. Originally considered different from acute GVHD simply by time kinetics, later onset, a more protracted course and less acute mortality, it became clear that cGVHD was an entirely separate clinical syndrome. Even though acute and cGVHD have some of the same target organs, the organ manifestations of cGVHD are different, clinically resembling autoimmune and mixed collagen vascular diseases, immunologically showing an array of abnormalities leading investigators to coin the phrase of "disordered immunity" to categorize the syndrome at least descriptively.

After animal models of cGVHD were developed, that mimick the human disease, a more precise description is emerging clinically, pathologically, and immunobiologically pointing towards a pathophysiology entirely different from acute GVHD. A rat model of cGVHD can serve as a prime example. After allogeneic mismatched marrow transplantation, rats will spontaneously develop a syndrome of alopecia, weight loss, Sjogren's syndrome, systemic sklerosis and chronic hepatitis between 6 months and 1 year after grafting. Histologically lichen planus, dermal sklerosis, dacryosialoadenitis, and periportal mononuclear cell infiltration with piecemeal necrosis of the liver are present. Immunologically the animals show a combined immunodeficiency with severe impairment of T cell function as well as the presence of large numbers of alloantigen non-specific suppressor T cells in spleen and blood. Spleen cells from rats with cGVHD will adoptively transfer the syndrome to irradiated secondary recipients, incubation of the inoculum with anti-T cell antibody and complement will prevent the transfer. Enrichment of normal marrow inocula with non-specific T cell suppressors prior to transfer into irradiated rats will cause cGVHD within 4 weeks. Adoptive transfer is possible to all tested rat strains even to those syngeneic to the original donor. These rapidly induced models show that immunoglobulin deposits in the skin, vasculitis and antinuclear antibodies appear to be epiphenomena, whereas the presence of non-specific suppressor T cells and certain thymus abnormalities (hypocellular medulla with prominent epithelium) are obligatory. Although the causality of suppressor T cells appears a firm assumption, the association with a thymic defect appears equally important. Adoptive transfer is possible in irradiated recipients and only if the thymus is in the radiation field. If the thymus is shielded the adoptive transfer of cGVHD is not possible. This suggests that a radiosensitive thymic control system is present which, when impaired, permits the disequilibrium, the expansion of non-specific suppressor T cells and the establishment of cGVHD.

Cytomegalovirus and Interstitial Pneumonia

P18 VIRAL INFECTIONS IN MARROW TRANSPLANT RECIPIENTS Joel D. Meyers, M.D. Fred Hutchinson Cancer Research Center, Seattle, WA 98104

Viral infections are frequent and often severe after marrow transplantation, related both to epidemiologic or immunologic factors which increase exposure to or reactivation of viruses and to the profound and prolonged immunodeficiency which increases the severity of viral infection. Although infections with adenoviruses and papovaviruses, and occasionally with RNA enteric or respiratory viruses, may occur, the most common and severe infections are due to members of the herpesvirus family. These infections have characteristic epidemiology and clinical manifestations, although atypical presentations may also occur.

Infection due to herpes simplex virus (HSV) occurs before or soon after transplant in up to 80% of patients who are seropositive before transplant. Although most patients develop either oral or genital mucosal infection, both gastrointestinal and pulmonary HSV infection may occur through contiguous spread of infection. HSV esophagitis was particularly common in the past. Viremic HSV infection with dissemination to multiple organs has also occurred, but is rare. HSV infection can now be controlled through the therapeutic or prophylactic use of acyclovir. As a result HSV has markedly decreased in significance although multiple recurrences of infection among patients with GVHD, and infection with acyclovir-resistant HSV may still occur. Varicella-zoster virus (VZV) infections occur latest after transplant, with the median onset in the 5th month after transplant. Preferred treatment for VZV infection is acyclovir, which is superior to vidarabine both in the prevention of virus dissemination and in accelerating local healing. Sequelae such as local scarring, post-herpetic neuralgia, or other neurologic syndromes may still occur even among treated patients. Without treatment, the mortality rate of VZV infection is 5-10%. Least is known about Epstein-Barr virus (EBV) infection, although many cases of EBV-related lymphoproliferative disease have now been reported. Other manifestations such as hepatic dysfunction and perhaps neutropenia may also be related to EBV infection, although little information is available.

The most important virus remains cytomegalovirus (CMV), which causes pneumonia in approximately 15% of allogeneic recipients with a 85% mortality rate. Other important syndromes include gastrointestinal disease due to CMV, and CMV retinitis which is uncommon in marrow transplant recipients. Prevention of primary CMV infection in seronegative patients through use of solely seronegative blood products is clearly effective. Passive immunoprophylaxis with globulin remains of unclear benefit and continued controversy. Treatment of established CMV infection, and prevention of virus reactivation among seropositive patients, remain elusive although initial trials with 9-[2-Hydroxy-1-(Hydroxymethyl)-Ethoxymethyl]Guanine show promise that effective treatment or prophylaxis may be available in the future.

P19 RISK FACTORS FOR INTERSTITIAL PNEUMONIA. Roy S. Weiner, Mortimer Bortin, and Alfred Rimm. Division of Medical Oncology and Bone Marrow Transplantation Program, University of Florida, Gainesville FL 32610 and International Bone Marrow Transplantation Registry (IBMTR), Medical College of Wisconsin, Milwaukee, WI 53226.

Interstitial pneumonia (IPn) is a major cause of morbidity and mortality for patients undergoing allogenic bone marrow transplantation. Of 3312 allografted patients in the IBMTR database, the overall incidence of IPn is 22%. A trend analysis of IPn from 1978 through 1984 reveals a decreasing incidence for both leukemia and severe aplastic anemia (SAA). Cytomegalovirus is associated with almost 40% of all cases. The case fatality rate is greater than 80%. An analysis of 932 patients allografted for leukemia with bone marrow from histocompatible siblings identified six independent factors which influence the risk of IPn.[1] Older patients are at greater risk than younger patients; patients with Karnofsky performance ratings 100% are at greater risk than patients with 100%; patients transplanted 6 months from diagnosis are at greater risk than patients transplanted earlier; patients with more severe acute graft vs host disease (GvHD) are at greater risk than patients with less GvHD; patients who receive Methotrexate for prophylaxis against GvHD are at greater risk than those who receive Cyclosporin A; and for those patients who receive Methotrexate, the risk increases if their total body irradiation is delivered at a dose rate 4 cGy per minute. A preliminary analysis of risk factors among 439 patients with SAA allografted with histocompatible sibling bone marrow reveals an overall incidence of 19% compared with 29% in allografted leukemia patients. Age, severity of AGvHD, the use of Methotrexate for GvHD prophylaxis, and the use of irradiation, especially total body irradiation, are associated with an increased risk of IPn in allografted SAA patients. Defined factors in patient selection and management appear to influence the risk of IPn. The factors associated with increased IPn contribute to understanding the pathogenesis of the disease. Patient factors such as age and defined elements of the transplant regimen appear to have greater impact on the risk of IPn then does the patient's underlying disease. These factors should serve as foci for studies designed to decrease the incidence of IPn and the attendant morbidity and mortality.

1. Weiner, R.S., Bortin, M.M., Gale, R.P., Gluckman, E., Kay, H.E.M., Kolb, H.J., Hartz, A.J., and Rimm, A.A.: Interstitial pneumonitis following bone marrow transplantation: Assessment of risk factors. Annals of Internal Medicine, In Press 1985.

Autotransplantation

P20 BONE MARROW TRANSPLANTATION FOR POOR PROGNOSIS NEUROBLASTOMA, R.C. Seeger[1], J. Wells[2], C. Lenarsky[1], S.A. Feig[1], M. Selch[3], T.J. Moss[1], J. Ugelstad[4], and C.P. Reynolds[5]. Depts. of Pediatrics[1], Medicine[2], and Radiation Oncology[3], UCLA School of Medicine, Los Angeles, CA 90024; University of Trondheim, Trondheim, Norway[4]; Naval Medical Research Institute, Bethesda, MD 20814[5].

Approximately 60% of patients with neuroblastoma have only a 10% chance of tumor-free survival if treated conventionally. We have investigated intensive chemoradiotherapy (VM-26, adriamycin, melphalan, CDDP and total body irradiation; VAMP-TBI) followed by allogeneic or autologous bone marrow transplantation (BMT). Thirteen patients were transplanted before developing progressive disease (PD) and 7 afterwards; 12 received autologous and 8 received HLA identical marrow. Four autologous marrows were treated ex vivo to remove tumor cells, whereas 8 were untreated. Ex vivo treatment combined sedimentation, filtration, and monoclonal antibodies with magnetic beads. All autologous marrows were evaluated with immunoperoxidase staining, which can detect 1 tumor cell among 100,000 normal cells. Outcome for patients transplanted after having developed PD was as follows: 3 toxicity related deaths, 1 death from brain hemorrhage, and 3 relapses before 9 mos after BMT. In contrast, outcome for the 13 patients who had not developed PD was encouraging: 7 are tumor-free survivors from 1+ to 37+ mos after BMT; 4 patients relapsed, 1 had a toxicity related death, and 1 died of sepsis at home; estimated disease-free survival at 37+ mos is 45%. Our current study, which is limited to patients who have not developed PD, employs more aggressive induction chemotherapy and ex vivo treatment of all autologuos marrow. These modifications may further increase the percentage of patients with tumor-free survival.

P21 INTENSIFICATION WITH HIGH DOSE CHEMOTHERAPY AND AUTOLOGOUS BONE MARROW TRANSPLANTATION IN RESPONDING SOLID TUMORS. Gary Spitzer and Karel A. Dicke, Department of Hematology and Bone Marrow Transplantation, University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030.

The use of high dose chemotherapy (HDC) intensification (INT) with autologous bone marrow support (ABMS) has been explored by our group in more than 50 patients over the last 5 years. The majority of patients treated showed a partial or complete remission before intensification. All patients received 2 courses of cyclophosphamide (4.5gm/m2) and VP-16 (600-750mg/m2) with ABMS 4 to 5 weeks apart as the major component of therapy. The addition of Adriamycin and methotrexate to this program caused significant gastro-intestinal toxicity; the recent addition of cis-diamine dichloride platinum (C-DDP) (100-150mg/m2) appears to have tolerable and predictable toxicity. Despite the advanced age (median 59 years) and the occurence of serious infections, not one patient has died during INT. Six of the thirty-two limited stage small cell bronchogenic carcinoma patients are alive and disease free (DF) off therapy for 4+ years. Long term DF survival was greatest in those patients INT in CR and receiving maximal INT (2 vs. 1 INT). Future aims are (1) identify the maximal tolerated doses of cyclophosphamide, VP-16 and C-DDP in combination in both young and old (the latter being over 45 years) patients, (2) determine if INT in partial remission with this triple drug combination can cause prolonged DF survival and (3) evaluate if this HDC combination is of value for responding (partial remission and complete remission) solid tumor patients regardless of histological type.

Genetic Disorders: New Directions

P22 **TRANSPLANTATION IN METABOLIC AND GENETIC DISORDERS**

A J Barrett, Westminster Hospital, Dean Ryle Street, London SW1, UK

It has been estimated that there are about 90 congenital disorders potentially correctable by bone marrow transplantation (BMT)[1]. Over 175 patients have received BMT for congenital disorders - over 70 for thalassaemia. Disorders for which BMT has been attempted can be classified as follows:

1. Defects directly associated with abnormalities in one or more myeloid or lymphoid cell lines, eg haemoglobinopathies, granulocyte defects, hereditary platelet defects, immune deficiency disorders such as Wiskott Aldrich disease, and combined immune deficiency.
2. Defects in macrophages or osteoclasts in the reticulo-endothelial system, eg Gaucher's disease and osteopetrosis.
3. Defects where the major abnormality is outside the reticulo-endothelial system where bone marrow transplantation may provide a missing enzyme to correct the metabolic defect in the tissues, eg mucopolysaccharidoses.
4. Defects where the major abnormality is outside the reticulo-endothelial system where it is not certain whether BMT can replace missing enzymes, eg gangliosidoses, and glycogen storage disease.

Preclinical animal models for BMT in inborn errors in metabolism are limited in their usefulness because of the paucity of well defined metabolic defects in experimental animals and because it is unclear how closely the experimental model relates to the human disease. As a consequence, little is known about the efficacy of enzyme replacement treatment which may be impaired by premature degradation of the transplanted enzyme, and lack of penetration in remote tissues, such as the central nervous system. It is only by clinical transplants that the answer to some of these problems will be known. In addition there are several specific problems with BMT in genetic disorders which may limit its success:

1. Pre-existence of irreversible damage to major organs before the transplant.
2. Difficulty in obtaining satisfactory engraftment in patients with normally proliferating bone marrow.
3. The relatively young age of potential transplant recipients decreases the possibility of finding matched sibling donors in the family. This has encouraged some groups to attempt mismatched parental BMTs but the efficiency of bone marrow derived from obligate parental heterozygotes for correcting the disease is not well defined.

Updated results from the International Bone Marrow Transplant Registry in a wide variety of genetic disorders will be presented.

Reference

Hobbs J R. Bone marrow transplantation for inborn errors. Lancet 2, 735, 1981.

P23 BONE MARROW TRANSPLANTATION IN THALASSEMIA AFTER BUSULPHAN AND CYCLOPHOSPHAMIDE. REPORT ON 70 CASES.Lucarelli G.,Galimberti M.,Polchi P., Delfini C.,Giardini C.,Baronciani D.,Politi P.,Manenti F.,Divisione Ematologica di Muraglia, Centro Trapianto Midollo Osseo, Ospedale di Pesaro,Pesaro, Italy.

From June 1983 70 patients with beta-thalassemia have been transplanted in Pesaro after receiving 14mg/Kg of busulphan and 200 mg/Kg of cyclophosphamide. 40 patients aged between 1 and 8 years (Group 1) received methotrexate for GVHD prophilaxis. Of 30 patients aged between 8 and 14 years (Group 2) eigth received methotrexate and 22 ciclosporine for GVHD prophilaxis. In Group 1 A-GVHD was 85% Grade 0-2 and 15% Grade 3-4. In Group 2 A-GVHD was 78% Grade 0-2 and 22% Grade 3-4. A-GVHD was the cause of death in 3 patients in Group 2.

Four patients died in Group 1 and six died in Group 2. Three patients in Group 1 and 1 in Group 2 are alive with thalassemia after the transplant. 33 patients in Group 1 and 23 in Group 2 are alive and well with functioning graft. Overall results are: 14% dead, 6% alive with thalassemia, 80% alive and well with functioning graft.

Allotransplants in Leukemia, and Autotransplants in Leukemia

P24 PRELIMINARY RESULTS OF A NEW BUSULFAN-CYCLOPHOSPHAMIDE REGIMEN FOR ALLOGENEIC BONE MARROW TRANSPLANTATION IN LEUKEMIA, Edward Copelan and Peter Tutschka, The Ohio State University Bone Marrow Transplant Program, Columbus, Ohio, 43210.

The toxic and therapeutic effects of busulfan (16 mg/kg) and cyclophosphamide (120 mg/kg) with allogeneic BMT were evaluated in 35 patients. Three patients had preleukemia, 7 ANLL in remission, 6 primary refractory ANLL or ANLL in relapse, 1 ALL in remission, 4 ALL in relapse, 3 CML in chronic phase and 11 CML in accelerated or blastic phase. The median duration of neutrophil counts < 500 was 9 days. Fourteen patients did not require antibiotics. Unsupported platelet counts < 40,000 occurred for a median of 14 days. Nausea, vomiting and diarrhea occurred frequently. Severe stomatitis occurred in only 2 patients, both in the presence of documented Herpes simplex infection. Transient liver function abnormalities occurred but only 1 patient developed venoocclusive disease. Patients were discharged a median of 22 days after transplantation. Two patients developed fatal interstitial pneumonia; no patient developed CMV pneumonia. Two patients died of infections >100 days following BMT. All patients transplanted with active disease had complete tumor responses. Four patients (none transplanted in remission) have relapsed. One of these patients died, 3 have undergone second transplants. Thirty of 35 patients are disease-free survivors following BMT. The median survival of all patients is 209 days (range 29 to 602 days). Of patients treated for preleukemia, acute leukemia in remission, and CML in chronic phase 14 of 14 are disease-free survivors a median of 390 days (range 69 to 602) post BMT. This regimen appears to have less toxicity than conditioning regimens generally used in BMT for leukemia and preliminary results suggest high antileukemic efficacy.

P25 EFFECT OF ACUTE GRAFT-VERSUS-HOST DISEASE (GVHD) ON OUTCOME OF BONE MARROW TRANSPLANTATION IN ACUTE NON-LYMPHOCYTIC LEUKEMIA (ANLL). SA Feig, ME Nesbit, J Buckley, B Lampkin, I Bernstein, T Kim, S Piomelli, J Kersey, P Coccia, RJ O'Reilly, C August, ED Thomas, and D Hammond. Children's Cancer Study Group (CCSG), Pasadena, CA 91101

Seventy-two matched sibling marrow transplants were performed on children with ANLL in first remission as part of a cooperative trial by CCSG (CCG-251). Most patients (53) were conditioned with cyclophosphamide and single-dose total body radiation; 18 received fractionated radiation and one patient was conditioned with busulfan and cyclophosphamide. Methotrexate (100 d) was used as prophylaxis against GVHD. Three patients had fatal sepsis prior to engraftment. Among 69 engrafted patients, 29 developed significant ($\geq$ grade II) acute GVHD. In this group, there were 11 cases of interstitial pneumonia (8 fatal), 5 cases of fatal sepsis, and no relapses. Three year actuarial survival is 55% in patients with significant acute GVHD. Nine of the 16 survivors in this group have developed chronic GVHD. Among 40 patients with clinically insignificant (< grade II) acute GVHD, there were 3 fatal cases of interstitial pneumonia, 1 fatal sepsis, and 11 relapses. In this group, 3 yr actuarial relapse-free survival is 58%, and the probability of relapse is 32%. Eight of 25 survivors in this group have developed chronic GVHD. For the entire group of patients, the 3 yr actuarial disease-free survival is 55% (95% C.I.: 43-67%). While acute GVHD did not influence ultimate survival, acute GVHD was associated with increased risk of early death (due to sepsis or interstitial pneumonia), and a decreased risk of recurrent leukemia.

P26 IMMUNOLOGIC PURGING IN AUTOLOGOUS BMT (ABMT): RELEVANCE OF TARGET ANTIGEN DENSITY ON BLASTS TO THE EFFICIENCY OF COMPLEMENT-MEDIATED CYTOLYSIS.

Grob, J-P., Campana, D., Timms, A., Coustan-Smith, E., Janossy, G., Prentice, H.G.
Royal Free Hospital, London.

Purging of remission marrow for ABMT is frequently performed in ALL using complement mediated cytolysis (C' lysis). To select the most efficient Mab for C' lysis in individual patients, the density of antigens (AgDens) present on blasts of 17 patients with ALL (10 c-ALL, 1 B-ALL, 6 T-ALL) was estimated and correlated to the efficiency of C' lysis. AgDens estimation in units of a panel of B- and T-lineage associated antigens was based on the intensity of fluorescence in flow microfluorometry using a standardised indirect immunofluorescence assay. Fluorescent microspheres were used as standard and given the value of 100 units (U). C' lysis was achieved using one incubation with single Mabs or Mab cocktails, followed by 2 incubations with rabbit (RC' lysis) or human autologous (HC' lysis) serum. AgDens varied widely for single antigens (8-71U) and did not correlate with the percentage of blasts expressing the antigen. With RC' lysis, 16/17 antigens with AgDens >32U allowed for the lysis of 96-99,99% blasts, while 0/9 antigens with AgDens <32U attained such efficiency ($p > 0.0001$). With HC' lysis, only 5 of 12 antigens with AgDens >32U were associated with >95% blast lysis. The use of Mab cocktains increased the efficiency of RC' lysis only if at least one of the Mabs constituting the cocktail induced efficient RC' lysis when tested alone (98% lysis).

P27 AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) IN ACUTE LEUKEMIA USING EX VIVO MARROW TREATMENT WITH 4-HYDROPEROXYCYCLOPHOSPHAMIDE (4HC). H. Kaizer, A. M. Yeager, H. G. Braine, M. Colvin, S. Rowley, R. Saral, and G. W. Santos. Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL 60612 and The Johns Hopkins Oncology Center, Baltimore, MD 21205.

One of the major problems with ABMT in acute leukemia is the potential presence of occult clonogenic leukemic cells in remission marrow. We have been studying the use of ex vivo treatment of remission marrow with 4HC to "purge" such occult cells and have recently reported the completion of a phase I study (BLOOD 1985;65:1504) demonstrating the maximum safe concentration of 4HC. There are now 28 patients with acute lymphoblastic leukemia (ALL) and 24 patients with acute non-lymphoblastic leukemia (ANLL) in second or subsequent remission who have been transplanted on the phase I or subsequent phase II study and are evaluable for outcome. Excluded from this analysis are patients receiving marrow treated at 4HC concentrations >100 ug/ml and 3 patients transplanted in initial remission. Preparative regimens consisted of cyclophosphamide (CY) and total body irradiation for patients with ALL and busulfan and CY for patients with ANLL. Actuarial analysis of relapse-free survival (RFS) for patients with ALL reveals a plateau on the RFS curve of <10%, a result which is inferior to reported results with syngeneic BMT. On the other hand, the currently estimated plateau for patients with ANLL is approximately 45%. Definitive conclusions will require longer durations of follow-up.

P28 GENOTYPIC ANALYSIS OF CELL POPULATIONS WITH HIGHLY POLYMORPHIC DNA PROBES. R.G. Knowlton, V.A. Brown, J.C. Braman, J.W. Schumm, D. Barker, J. Ritz*, and H. Donis-Keller, Collaborative Research, Inc., Lexington, MA, and *Dana-Farber Cancer Institute, Boston, MA.

The use of DNA markers known as restriction fragment length polymorphisms (RFLP's) has been shown to be a sensitive and informative method of distinguishing patient and allogeneic donor cells after bone marrow transplantation. To apply the test, it is necessary in each case to find DNA probes which display both patient-specific and donor-specific bands in Southern transfer hybridization. Because the recipient and donor are usually closely related, more than one highly polymorphic marker is required. We have isolated a set of 13 cloned DNA's from very highly polymorphic loci by which even siblings can usually be distinguished. With just five of these probes, we can distinguish the genotypes of the patient and a sibling donor in more than 99% of cases (except between identical twins). The availability of many highly polymorphic probes allows us not only to test virtually every transplant case, but also to select a probe which can detect both the patient and donor-specific bands in a single hybridization with optimal resolution and sensitivity. We have applied these probes to the analysis of cells from peripheral blood and bone marrow after transplantation and demonstrated their usefulness in confirming engraftment of donor cells or graft rejection, and in detecting mixed lympho-hematopoietic chimerism prior to leukemic recurrence.

P29 AUTOLOGOUS TRANSPLANTATION OF BLOOD-DERIVED HEMOPOIETIC STEM CELLS: COMPLETE HEMOPOIETIC RECONSTITUTION AFTER MYELOABLATIVE TREATMENT, Martin Körbling, Hans Martin, Bernd Dörken and Werner Hunstein, Medizinische Poliklinik, University of Heidelberg, 69 Heidelberg, West Germany

Hemopoietic engraftment following myeloablative therapy can be achieved with human peripheral blood cells as demonstrated by successful autotransplantation in patients with CML. We report a patient with stage III Burkitt's lymphoma who provides clear evidence that complete and permanent hemopoietic reconstitution can be achieved after myeloablative treatment using his own cryopreserved circulating stem cells. Following autologous blood stem cell transplantation all cell lines reappeared very rapidly, i.e. 1.000 leucocytes/ul 9 days post transplant, 500 granulocytes/ul and 50.000 platelets/ul 10 days thereafter. B-cells reached normal values around day 35 post transplant.The high yield of hemopoietic precursor cells harvested from this patient by multiple cytapheresis, cryopreserved and eventually retransfused (2.1×10^5 CFU-GM/kg) reflects an expansion of the blood stem cell pool at the time of stem cell harvest. Since cytapheresis started just two weeks after completion of chemotherapy (COMP), stem cell harvest was probably performed during a chemotherapy induced blood stem cell overshoot.
To find out optimal conditions for blood stem cell harvest additional preliminary data will be presented on multiple stem cell collections from patients with advanced Hodgkin's disease including prior radiation to the pelvic site.Various approaches of inducing stem cell overshooting as a consequence of transient myelosuppression will be discussed.

P30 AUTOLOGOUS BMT FOR ACUTE MYELOID LEUKAEMIA : CLINICAL USE OF MARROW GROWN IN LONG-TERM CULTURE SYSTEM. G.R. MORGENSTERN, J. CHANG, T.M. DEXTER, J.H. SCARFFE, L. COUTINHO, N.G. TESTA.

When long-term cultures are established from marrow cells of leukaemic mice there is a preferential loss of leukaemic cells and the surviving normal cells can be used to perform a marrow transplant with disease-free long-term survival. We have exploited this system for the growth of human marrow cells and have shown that leukaemic cells from a patient with AML,with a 16q- chromosome marker, were non-detectable following in vitro culture for 7 to 14 days. This result encouraged us to use long-term cultured marrow cells to treat young adults with AML who lack an HLA-compatible donor. Bone marrow is harvested after induction and consolidation chemotherapy and used to establish long-term cultures.

The patients undergo conditioning with Cyclophosphamide followed by fractionated TBI. After 10 days the cultured marrow is collected and washed prior to reinfusion. Three patients have undergone BMT using this technique. The first (male aged 15) was in frank relapse (60% blasts) with a 16q- karyotype at the time of harvest and BMT. He reconstituted with a complete remission (CR) marrow with loss of the chromosome abnormality and normal peripheral blood indices. He relapsed 30 weeks after BMT and is currently well undergoing reinduction 32 weeks after BMT. The subsequent patients (females aged 18,35) were both in first CR at time of harvest and BMT. They are well and in remission at 28,13 weeks post BMT.

These results indicate that marrow grown in long-term culture for 10 days can be successfully used to reconstitute haemopoiesis and that preferential survival of normal versus leukaemic stem cells may offer a way of reducing leukaemic relapse after autologous BMT.

P31 APPLICATION OF MONOCLONAL ANTIBODIES FOR AUTOLOGOUS BONE MARROW TRANSPLANTATION IN NULL CELL TYPE ACUTE LYMPHOBLASTIC LEUKEMIA, Yasuo Morishima, Hiroshi Sao, Ryuzo Ueda, Satoshi Yoshikawa, Yoshihisa Kodera, Ryuzo Ohno and Toshitada Takahashi, 1st Dept. of Int. Med. Nagoya Univ., Aichi Red Cross Blood Center, Aichi Cancer Center Res. Inst., Nagoya 1st Red Cross Hosp., NAGOYA, Japan

Autologous bone marrow transplantation (BMT) in null cell type acute lymphoblastic leukemia (Null-ALL) was carried out with the depletion of leukemia cells from transplanted bone marrow.

Patients' autologous bone marrow cells, which had been harvested during remission period, were treated with complement and three monoclonal antibodies (NL-1, NL-22 and HL-47) reactive to null ALL cells produced in our laboratories. Five patients were transplanted during the first remission period after preconditioning with intensive chemotherapy and total body irradiation, while the other two patients were transplanted during the fourth remission period and third relapse, respectively, in poor clinical condition. The good preservation of hematopoietic stem cells after the antibody treatment and cryopreservation of bone marrow cells were demonstrated in all the seven cases studied. Clinically, prompt recovery of white blood cells and platelets were observed in the five patients who received BMT during the remission period, and four among these have continued remission (18, 6, 3 and 2 months). These results suggested that these three antibodies can be utilized for autologous BMT in Null-ALL patients.

P32 ANTITHYMOCYTE GLOBULIN (ATG) MAY ALLOW ENGRAFTMENT OF T-DEPLETED MARROW IN HAPLOIDENTICAL BONE MARROW TRANSPLANT (BMT) RECIPIENTS. S. Neudorf, M. McGill, B.Lampkin, L. Jenski, J. Sambrano, D. Hake, R. Harris; Children's Hospital Medical Center and Hoxworth Blood Center, Cincinnati, Ohio

T cell depletion of donor marrow can prevent graft-vs-host disease (GVHD) in patients (pt) with matched or mismatched donors. Graft failure, however, is common especially in mismatched pts. Animal models suggest that natural killer (NK) cells cause graft rejection. We therefore asked whether therapeutic concentrations of ATG could eliminate NK activity in vitro and whether administration of ATG to mismatched BMT recipients could prevent graft rejection. Normal peripheral blood mononuclear cells were treated for 45 min with ATG (UpJohn) plus 10% plasma as a source of complement prior to incubation with K562 cells (NK target) for 6 hrs. In 2/2 lots tested, $\geq$ 1.0 mg/ml eliminated > 95% of NK activity. ATG was evaluated clinically in 6/8 haploidentical BMT recipients with leukemia. Five pts received high dose Ara C (HDAraC) 3 gm/m^2 x 12 doses, ATG 30 mg/kg/day x 4 doses and total body irradiation (TBI). One pt received ATG, cyclophosphamide and TBI. Two pts received HDAraC, TBI and no ATG. All 8 received parental marrow depleted using soybean lectin and E rosetting. All 6 that received ATG engrafted on median day +25 (range 17-45 days) without GVHD. Two pts that did not receive ATG did not engraft and died. MLC reactivity and cell dose did not correlate with graft failure. Of pts that engrafted, 2 relapsed, 1 died of toxic epidermal necrolysis, and 3 died from viral infections. We conclude that the addition of ATG to BMT conditioning regimens may allow engraftment from haploidentical donors and hypothesize that the effects of ATG may be due to elimination of NK activity.

P33 **AUTOLOGOUS BONE MARROW TRANSPLANTATION (BMT) FOR PATIENTS (PTS) WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL): USE OF TWO PREPARATIVE REGIMENS AND PURGING WITH BA-1, BA-2, BA-3 AND COMPLEMENT.** N. Ramsay, T. LeBien, T. Kim, A. Goldman, M. Nesbit, P. McGlave, D. Hurd, W. Woods, D. Weisdorf, J. Kersey, University of Minnesota, Minneapolis, MN 55455.
From 1982-1984, 28 pts with ALL underwent autologous BMT following conditioning with cyclophosphamide 60 mg/kg/d x 2 and fractionated total body irradiation at a dose of 165 cGy given BID x 4 days (10 cGy/min). All pts lacked matched donors and had experienced at least one on therapy relapse. Remission bone marrow was treated with the monoclonal antibodies BA-1, BA-2, BA-3 and complement. The pts ranged in age from 3-42 yrs (median=9 yrs) at BMT. Ten pts were in 2nd remission and 18 pts were in 3rd/4th remission. All pts engrafted. One pt died of infection at day 21. 21 pts relapsed from 1.4-9.1 mos (median=3.3 mos). Six patients are alive disease-free 21-40 mos (median=31 mos) post BMT for a disease-free survival of 21% ± 14% (95% confidence limits). Because of the high relapse rate, a subsequent study was initiated in 1985. 11 pts were transplanted with treated autologous marrow after conditioning with single dose total body irradiation at 850 cGy (26 cGy/min) and cytosine arabinoside 3 gms/m^2 BID x 6 days. The pts were 3-30 yrs (median=13 yrs) at BMT. All pts engrafted, however, 1 pt died of infection 41 days post transplant. Four pts have relapsed at 2.7-3.5 mos and 6 pts remain disease free from 3-9 mos after BMT. Although the follow-up is short, no differences are observed between the two regimens at this time. The relapse rate is high following autologous BMT, however, certain poor risk pts experience long term survival. The high relapse rate may be ascribed to inadequate conditioning, purging and/or lack of graft versus leukemia effect in these pts receiving autologous BMT.

P34 TIMING OF MARROW TRANSPLANTATION IN CHRONIC MYELOGENOUS LEUKEMIA (CML). G.B. Segel, M.A. Lichtman, and W. Simon. U. of Roch. School of Medicine, Rochester, N.Y.
Only marrow transplantation from an allogeneic sibling offers the potential of cure of CML. The timing of the marrow transplant is made difficult by 1)the high peritransplant mortality of 20 to 35% and 2)a group of patients with prolonged chronic phase of CML which can be identified on the basis of prognostic indices (% blood myeloblasts, spleen size, and platelet count). We have developed a mathematical model and computer program which consider the patient's age, the prognostic indices and the cure rate by transplantation to assess the risk of delaying the transplant. The computation does not offer the option of avoiding transplantation since long term survival ultimately requires transplantation. Three prognostic groups were considered as described by Sokol and coworkers (Blood 63:789, 1984) (I-best, II-intermediate, III-worst prognosis). Using this computer program the ratio of the calculated life expectancy to the normal life expectancy (CLE/NLE) indicated, for example, that a delay of one year prior to transplantation posed little additional risk to overall survival, i.e. fall in CLE/NLE, in 20 year old patients in groups I and II. The CLE/NLE ratio fell at an accelerated rate after six months for those patients in group III indicating that early transplantation was preferable in this group. For those patients 40 years of age it was no longer clear that even group III patients should be transplanted within the first six months. Although transplantation should eventually be performed in all patients for whom it is available to achieve a normal life span, this computer program optimizes the time of transplantation by considering patient age, prognostic group and cure rate by transplantation.

P35 DYE-MEDIATED PHOTOLYSIS OF TUMOR CELLS: IMPLICATIONS FOR AUTOLOGOUS BONE MARROW TRANSPLANTATION, Fritz Sieber, Midwest Children's Cancer Center, Department of Pediatrics, Medical College of Wisconsin, Milwaukee, WI 53233
In this communication we report on several preclinical applications of dye-mediated photolysis for the purging of simulated autologous remission marrow grafts of residual tumor cells. The potential-sensitive lipophilic dye, merocyanine 540 (MC 540), is avidly bound by leukemia and neuroblastoma cells. If these cells are simultaneously exposed to light, they are rapidly killed. By contrast, pluripotent hematopoietic stem cells are inactivated very slowly, presumably because they have a low affinity for dye molecules. We exploited this marked difference in photosensitivity to selectively inactivate L1210 and P388 leukemia and Neuro 2a and NB41A3 neuroblastoma cells in mouse bone marrow grafts. Lethally irradiated mice that were transplanted with photosensitized 100:1 mixtures of normal marrow cells and tumor cells survived and remained free of disease. In vitro clonal assays indicated that the same approach may also be applicable to the purging of human marrow grafts. MC 540-mediated photosensitization reduced the concentration of HL-60, K562, Raji and Daudi leukemia cells 50,000-fold or more and the concentration of IMR-32, SK-N-SH and SK-N-MC neuroblastoma cells 500-33,000-fold, but preserved 52% of pluripotent hematopoietic progenitor cells (CFU-GEMM). These results suggest that dye-mediated photosensitization may be a promising approach to the extracorporeal purging of autologous remission marrow grafts. Supported by Grants AM27157, HD15311, and CA42734, from the NIH. F.S. is a Leukemia Society of America Scholar.

P36 ACUTE RENAL FAILURE ASSOCIATED WITH AUTOLOGOUS BONE MARROW TRANSPLANTATION. D.M. Smith, D.D. Weisenburger, W.P. Vaughan, and J.O. Armitage, University of Nebraska Medical Center, Department of Pathology and Microbiology and Internal Medicine.
A review of 33 consecutive autologous bone marrow transplants (BMT) revealed three cases of acute renal failure which developed immediately following reinfusion of cryopreserved bone marrow, and which could not be explained on the basis of hypotension or nephrotoxic drugs. Gross hemoglobinuria was noted in all autologous BMT patients, and may have contributed to the acute renal failure seen in the three patients. Histologic examination of the kidneys of one patient who died three days after BMT showed markedly dilated renal tubules filled with hemoglobin casts. The kidneys of the other two patients who died 8 and 20 days after BMT showed renal tubular necrosis and regeneration with numerous hemoglobin casts. All three patients had systemic candidiasis at autopsy and there is evidence that it was present at the time of BMT. Renal failure following infusion of hemolysate has been well described although the mechanism is not well understood. There is some evidence that stomal elements trigger disseminated intravascular coagulation (DIC) which results in tissue ischemia. Fungal sepsis may have predisposed these patients to develop renal failure by promoting DIC. We recommend that procedures which minimize the hemolysate content of cryopreserved bone marrow be used. If renal failure develops following reinfusion of autologous marrow, one should suspect systemic candidiasis.

P37 NOVEL LEUKEMIC PROGENITOR CELL ASSAYS TO OPTIMIZE AND STANDARDIZE EX VIVO MARROW PURGING IN AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) FOR ACUTE LYMPHOBLASTIC LEUKEMIA (ALL), Fatih M. Uckun, Kazimiera Gajl-Peczalska, Tucker W. LeBien, John H. Kersey, Dorothea E. Myers, Lou L. Houston and Daniel A. Vallera, Departments of Therapeutic Radiology, Laboratory Medicine/Pathology, Pediatrics and the Bone Marrow Transplantation Team, University of Minnesota, Minneapolis, MN 55455; Department of Biochemistry, University of Kansas, Lawrence, KS 66045.

Leukemic relapse which remains a major cause of treatment failure after reinfusion of purged autologous marrow grafts in ABMT for ALL could be due to residual refractory leukemia or to inadequately purged marrow. We have developed novel progenitor cell assays and evaluated the anti-leukemic efficacy of pokeweed antiviral protein or intact ricin containing immunotoxins, in vitro active cyclophosphamide derivatives such as 4-HC and ASTA Z 7557, monoclonal antibodies BA-1,2,3 plus complement as well as combined immunochemotherapy against blast progenitor cells freshly obtained from ALL patients. Our studies in 35 common B-lineage ALL and 25 T-lineage ALL patients revealed a marked heterogeneity among clonogenic ALL blasts with respect to their immunophenotype as well as toxin, complement and drug sensitivity. These preliminary studies clearly demonstrated that both pharmacological and immunological purging methods can effectively eliminate clonogenic ALL blasts in a significant number of patients and that a combination of both modalities will likely prove more effective than either modality alone.

P38 ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT) FOR HIGH RISK ACUTE LYMPHOBLASTIC LEUKEMIA (ALL): PRE-BMT PROGNOSTIC FACTORS PREDICT IMPROVED DISEASE-FREE SURVIVAL. DJ Weisdorf, ME Nesbit, NKC Ramsay, PB McGlave, WG Woods, DD Hurd, T Kim, A Goldman, J Kersey. University of Minnesota, Minneapolis, MN.
77 patients (pts) with high risk ALL underwent allogeneic BMT in remission (CR) between 8/78 - 9/85. High risk pts included those $\geq$21 years in 1st or greater marrow CR; 16 to 20 years in 1st CR with diagnostic WBC $\geq$10,000/ul, T- or B-cell phenotype, or Ph[1]; $\leq$20 years in $\geq$2nd CR or 1st CR with subsequent CNS relapse. 15 pts received cyclophosphamide (Cy) + moderate dose cytarabine, VM-26 and TBI (4 fractionated); 40 received Cy + fractionated TBI; and 4 received TBI + high dose cytarabine. All received HLA-MLC compatible sibling donor marrow. The pts (42 M, 35 F) ranged from 3.6 - 47.7 years (median 12.8) at BMT. 16 were in 1st marrow CR; 43 in 2nd; 16 in 3rd; 2 in 4th. 25 had prior extramedullary leukemia. Diagnostic WBC ranged from 1-945 x 10^3/ul (median 16 x 10^3/ul). After 24 months median follow-up, 27 pts have relapsed at 2.5-18.5 months (median 7.3) and 45 have died, 24 following relapse. Actuarial disease-free survival at 2+ years is 33.4 $\pm$ 11.5% (95% confidence interval), and 29 survive relapse-free up to 6.8 years post-BMT. Comparison of consecutive treatment protocols revealed no differences in outcome. Longer disease free survival (DFS) was not predicted by age, remission number, nor GVHD. Diagnostic WBC $\geq$50,000/ul or pre-BMT extramedullary leukemia significantly and independently predicted shorter DFS ($p<.007$). Although promising, these results suggest that advances in allogeneic BMT for ALL will require even more effective anti-leukemic conditioning.

P39 BONE MARROW TRANSPLANTATION (BMT) FOR TREATMENT OF LYSOSOMAL STORAGE DISEASES. Chester B. Whitley, John H. Kersey, Bruce R. Blazar, Norma K.C. Ramsay, William Krivit, University of Minnesota Bone Marrow Transplantation Team, Minneapolis, MN 55455

Eight patients with lysosomal storage diseases have been treated by BMT at the University of Minnesota: mucopolysaccharidosis (MPS) I (3), MPS III (1), MPS VI (1), metachromatic leukodystrophy (MLD) (1), Gaucher disease I (1), Wolman disease (1). Six had HLA/MLC matched siblings and engrafted readily after preconditioning with busulfan and cyclophosphamide. One patient lost engraftment and was successfully engrafted after a second BMT with cyclophosphamide and irradiation. Two infants (Gaucher and Wolman) had haplotype mismatched donors and both died; however, all surviving patients have had donor levels of enzymatic activity and reduction of substrate. Clinical response remains the critical issue. The MLD patient, 15 months post BMT, is learning new skills and attending kindergarten in contrast to her affected sibling who had, at this age, become comatose and decerebrate. The Hurler patients have improved dramatically in general health and have continued to progress in developmental skills. The MPS VI patient, now over three years since BMT, has continued to be stable with no recurrence of cardiopulmonary system pathology as noted before. The MPS III patient is 100 days post BMT and has engrafted. Graft-versus-host was a significant problem in two of the engrafted patients. From the data available from our survey and elsewhere, we can conclude that the continued careful use of BMT for lysosomal storage disease patients has proven to be clinically worthwhile.

Alternate Donors for Transplants, and Prevention of GvHd

P40 A POSITIVE ROLE FOR GRAFT-VERSUS-HOST REACTIVITY IN ENGRAFTMENT AND THE ESTABLISHMENT OF IMMUNOCOMPETENCE, Louise T. Adler and Frank L. Adler, St. Jude Children's Research Hospital, Memphis, TN 38101

Evidence obtained using an animal model suggests a positive role for graft-versus-host reactivity (GVHR) in engraftment and the establishment of immunocompetence. We have demonstrated the suitability of using outbred rabbits, matched for MHC (RLA) antigens and mismatched for Ig allotypes for transplantation studies. While permanent establishment of T cells from the donor cannot be demonstrated in this model, a helper function for such T cells in the establishment of B cell chimerism has been observed. Antibody responses of adult chimeras to test antigens are almost entirely of host origin unless the donor had been primed. This suggests that the failure of unprimed donor cells results from gaps in the repertoire. Most newborn RLA-heterozygous recipients of RLA-homozygous parental type lymphoid cells succumb to fatal GVHD. Those that survive differ strikingly in 2 ways from chimeras formed by the transfer of RLA-compatible cells. (1) Their hemopoietic systems are often completely reconstituted by B and T cells of donor origin, whereas recipients of RLA-matched cells always have mixed donor-host chimerism, and (2) donor lymphocytes in survivors of GVHD respond vigorously to test antigens without a need for donor priming. These findings suggest that complete removal of alloreactive cells from lymphoid cell transplants may have detrimental effects on engraftment and the rapid recovery of immunocompetence.

P41 GRAFT-VERSUS-HOST DISEASE (GVHD) IS ASSOCIATED WITH AUTOIMMUNE-LIKE THROMBOCYTOPENIA Claudio Anasetti, Keith Sullivan, Joachim Deeg, and Sherrill Slichter, Fred Hutchinson Cancer Research Center and Puget Sound Blood Center, Seattle, WA 98104

Persistent thrombocytopenia following allogeneic bone marrow transplantation (BMT) has been associated with poor patient survival. To understand the mechanism of the thrombocytopenia, we studied platelet and fibrinogen kinetics and antiplatelet antibodies in 20 patients between 60 and 649 days (median 90) after BMT. Three had pancytopenia, due to graft failure in two and to leukemic relapse in one. Seventeen had isolated thrombocytopenia (<100 x 10^3 plts/ul): marrow cellularity was either normal (5) or slightly reduced (12) and never was there a discrepancy between thrombopoiesis and myelo-erythropoiesis. All studies were performed with either autologous or marrow donor platelets. Platelet survival did not correlate with fibrinogen survival or splenic pooling, suggesting that disseminated intravascular coagulation and hypersplenism were not likely mechanisms of thrombocytopenia. Antiplatelet antibodies bound to autologous or marrow donor platelets were present in 5 of 12 patients studied. Antibody positive patients had lower platelet counts (30 ± 10 x 10^3/ul vs 49.1 ± 28.7, $p < 0.02$, t test) and survivals (1.32 ± 0.92 days vs 3.58 ± 2.02, $p < 0.001$, t test). Platelet bound autoantibodies were present in 5 of 6 patients with grade II-IV acute or chronic GVHD but were not present in 6 patients free of GVHD ($p < 0.01$, Fisher's exact test). We conclude that patients with acute or chronic GVHD may develop antiplatelet antibodies and autoimmune-like thrombocytopenia.

P42 CD5+ B CELLS ARE A MAJOR BLOOD CELL POPULATION AFTER HUMAN MARROW GRAFTING, J.H. Antin, K.A. Ault, J.M. Rappeport and B.R. Smith, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, 02115. We examined the recovery of blood B lymphocytes in 45 patients who underwent BMT. Two populations of recovering B cells were found by dual parameter immunofluorescence: (1) conventional B cells: CD5- (Leu1-), B1+, Leu12+, sIg+, and HLA-DR+; and (2) CD5+ B cells: CD5+, B1+, Leu12+, IgD+, IgM+, and HLA-DR+. CD5+ B cells are rare in normal adults but common in human fetal spleen (30-50%) and comprise the usual phenotype of chronic lymphocytic leukemia cells. We have shown previously that CD5+ B cells from human fetal spleen, unlike murine Ly-1+ B cells, are not constitutive producers of IgM. Polyclonal CD5+ B cells recovered beginning at day 30 after BMT, preceded the recovery of CD5- B cells, and often persisted for years ($>$ 5 years). In the absence of Grade 2 acute GVHD both B cell lines recovered rapidly and persisted. When patients with and without GVHD were compared, fewerCD5+ B cells were found in patients with aGVHD (6 ± 13 vs 149 ± 219 p=.04) and cGVHD (26 ± 42 vs 156 ± 226 p=.03), while CD5- B cells were decreased in aGVHD (5 ± 9 vs 176 ± 251 p=.03) but not cGVHD (76 ± 167 vs 174 ± 255 p=.15). Serum IgG levels correllated with the recovery of CD5- B cells ($p<.05$). Multivariate analysis showed no effect of prednisone or azathioprine independent of GVHD on the numbers of B cells. It is unknown whether CD5+ B cells reflect a stage of differentiation of normal B cells or a separate B cell lineage. We conclude that 1) B cells which express surface CD5 are common after BMT in the absence of GVHD, 2) conventional, CD5- B cells are more likely to recover during cGVHD than CD5+ B cells and their presence appears to be associated with recovery of serum IgG levels.

P43 CORRELATION BETWEEN T CELL DOSE IN T CELL DEPLETED MARROW INOCULA AND SUBSEQUENT ACUTE GRAFT-VERSUS-HOST DISEASE. K. Atkinson, M. Cooley, H. Farrelly, E.O'Flaherty J. Biggs, St. Vincent's Hospital, Sydney, Australia.

A correlation between the infused donor T cell number and severity of subsequent graft-versus-host disease (GVHD) in the recipient has been readily demonstrable in rodent models of bone marrow transplantation. We and others, however, have previously been unable to detect a correlation between the number of T cells present in unmanipulated HLA-identical sibling marrow inocula and subsequent GVHD. In the current study 12 patients with haematological malignancy received cyclophosphamide 60mg/kg on two consecutive days, fractionated total body irradiation 12Gy and an HLA-identical sibling marrow graft depleted of T cells by incubation with the pan T antibody anti HuLy-m1 (T11 equivalent) and rabbit complement. Residual T cell content was quantitated by flow cytometry and limiting dilution analysis (LDA). The median number (range) of T cells infused was 2.7 (0.5 to 56) x 10^5/kg recipient weight. Acute GVHD ranged from absent (Grade 0) to fatal (Grade IV). A correlation was present between the severity of acute GVHD and the number of T cells infused when quantitated by flow cytometry alone (r=0.44), by LDA incorporating the cloning efficiency derived from flow cytometric assessment of the marrow T cell content (r=0.44) and LDA alone (assuming 100% cloning efficiency) (r=0.42). A dose of 1 x 10^5 cells/kg recipient weight or less was associated with absent or minimal acute GVHD. These data demonstrate for the first time in man a relationship between the infused T cell dose and the degree of subsequent acute GVHD, suggesting that such monitoring can predict the severity of acute GVHD.

P44 QUANTITATION OF ENGRAFTMENT IN CELLS OF T-LINEAGE, B-LINEAGE, MYELOID AND ERYTHROID LINEAGE IN MURINE RECIPIENTS OF ALLOGENEIC OR SYNGENEIC MARROW GRAFTS, Bruce R. Blazar, Christine C.B. Soderling and Daniel A. Vallera, University of Minnesota, Minneapolis, MN 55455

We and others have used restriction fragment length polymorphisms as markers of allogeneic engraftment in man. Analysis of peripheral blood (PB) mononuclear cells (MNC) and neutrophils separately has demonstrated differences in the rate and extent of engraftment of these cells in recipients of T-cell depleted bone marrow (BM) grafts. We have now applied these same techniques to genotypically type murine graft recipients. Allogeneic marrow recipients of T-cell depleted or non-manipulated bone marrow were studied with H-2 phenotyping and/or were genotyped with Southern blotting techniques. Probes which hybridize to polymorphic sequences which were unique to the donor or recipient strain were used to distinguish engrafted donor cells from residual recipient cells. As compared to H-2 typing genotyping analysis was reliably more sensitive (1-10%), not dependent on the specific tissues studied, not influenced by host environment factors and was conclusive in the early post-transplant period. DNA genotyping is now being used to analyze the rate and extent of engraftment following cytoreduction and infusion of T-depleted or non-depleted BM. Recipient BM, thymus, lymph nodes, splenic MNC, PBMNC and neutrophils are now being analyzed. Preliminary data suggest that in recipients of non T-depleted donor marrow, PBMNC and splenic MNC engraft by d. +14 post-transplant with 0-10% thymic engraftment. DNA typing is further being used to quantitate engraftment of put-ative BM derived non-hematopoietic cells and in syngeneic recipients of sex-mismatched BM.

P45 B CELL FUNCTION AFTER T-CELL DEPLETED MARROW TRANSPLANTATION.

M.K. Brenner, J.Z.Wimperis, J.E. Reittie, A.V. Hoffbrand, Grob, J-P., Prentice, H.G.
Royal Free Hospital, London.

Specific T cell depletion is an effective way of preventing GvHD in the recipients of allogeneic marrow transplants. We have studied whether removal of mature T cells from the donor graft abrogates subsequent B cell function in the recipient. 38 donor/recipient pairs were divided into 4 groups: Group 1: donor alone was immunised one week pre-BMT with Tetanus Toxoid; Group 2: recipient alone immunised; Group 3: both received TT and Group 4 neither was immunised. When donors alone are immunised there is a short lived rise of antibody in recipient serum 18-28 days after BMT. This appears to represent Ab secretion by adoptively transferred B cells. When recipients alone are immunised, 2/7 recipients showed a similar short lived rise in Ab but delayed to 28-42 days after BMT. If both donor and recipient are immunised the Ab response is markedly enhanced in duration and titre. The Ab detected is actively synthesised in the recipient and not passively acquired from blood products since no rise in titre is seen if neither recipient nor donor are immunised. As T cells have been removed the source of help for these Ab responses is unclear. However, a study of the morphology, phenotype and function of natural killer cells in 18 of these patients showed i) NK/LGL numbers rapidly return to normal, ii) these LGL are activated and secrete IL-2, γIFN, BCDF, iii) such LGL can, in turn, activate and induce differentiation in autologous (donor) B cells, suggesting these cells have a significant role in inducing the antibody responses observed.

P46 SELECTIVE REMOVAL OF TOTAL T CELLS OR T CELL SUBPOPULATIONS FROM BONE MARROW WITH MONOCLONAL ANTIBODIES AND MAGNETIC IMMUNOBEADS. A Butturini, CP Reynolds, E Kedar, R Bjork, J Ugelstad, D Vo, R Seeger. Departments of Pediatrics and Microbiology and Immunology UCLA School of Medicine, Los Angeles, CA; Naval Medical Research Institute, Bethesda, MD; University of Trondheim, Trondheim, Norway.

Monoclonal antibodies (mab) and magnetic beads coated with goat anti-mouse Ig have been successfully used to remove total T cells or T cell subpopulations from human bone marrow and peripheral blood. Mab T1b, T3, T4, T8, T11, and T12, were tested either alone or in combinations. The extent of depletion was determined as follows: 1) by cell surface marker analysis (flow cytometry, immunoperoxidase staining, E rosette formation) on the day of depletion and after culturing cells for five days with IL-2 and PHA; 2) by T cell response to PHA, con-A or PWM; and 3) by limiting dilution analysis of cells cultured for 10 days with IL-2 and PHA. Total T cells were reduced three logs after one cycle of depletion with mabs T3, T11, T4 + T8 or all combined; with individual mabs, three logs of T8 cells and two logs of T4 cells were removed. Less than 10% of other cell populations were lost, and growth of CFU-G,M, natural killer cell activity, and immunoglobulin production were not effected. The high efficiency and selectivity of this procedure suggests that monoclonal antibodies and magnetic immunobeads will be suitable for removing either total T cells or T cell subpopulations in clinical trials of bone marrow transplantation. (We thank Coulter Immunology for monoclonal antibodies, Kirkegaard and Perry Labs for goat anti-mouse Ig, and Sintef for magnetic beads. Partially supported by NCI grant CA12800 and by the Concern Foundation.)

P47 FETAL LIVER TRANSPLANTATION IN DOGS. Gary R. Cain, Robert P. Gale, and Richard E. Champlin, Laboratory for Energy-Related Health Research, University of California, Davis, CA 95616 and Transplantation Biology Program, University of California, Los Angeles, CA 90024

We evaluated the ability of fetal liver hematopoietic cells to reconstitute hematopoiesis and immunity in lethally irradiated dogs. Seven dogs received DLA-identical grafts following 2-7.4 Gy fractions of total body irradiation (TBI). Three other dogs received grafts which were homozygous for a DLA haplotype shared by the recipient (e.g. AA->AB). Rapid and sustained engraftment was observed in all dogs and no post transplant immunosuppressive treatment was necessary. This conditioning regimen was insufficient, however, for preventing graft rejection in 5/5 dogs given DLA-mismatched grafts (e.g. AB->AA, BB->AA). A pretransplant conditioning regimen of 2-7.4 or 2-8.0 Gy TBI and cyclosporin A resulted in engraftment of 19 of 28 (68%) mismatched dogs. Restoration of hematopoiesis was rapid and complete. Return of immune function was comparatively delayed but sufficient to allow prolonged survival. Autoimmune syndromes occurred frequently following fetal liver transplantation, including immune thrombocytopenia (5 cases) and myasthenia gravis.

These data indicate that fetal liver cell transplantation can restore hematopoiesis and immunity in MHC mismatched recipients. Fetal liver may be an effective alternative to bone marrow transplantation into human patients who lack an HLA-identical donor.

P48 HLA-A,B,DR MATCHED, MLC-NONREACTIVE UNRELATED DONORS FOR BONE MARROW TRANSPLANTATION, Bruce M. Camitta, James T. Casper, Meg McElligott and Patrick G. Beatty, The Midwest Children's Cancer Center/Medical College of Wisconsin, The Blood Center of Southeastern Wisconsin (BCSEW) and the Fred Hutchinson Cancer Research Center.
Bone marrow transplantation is the treatment of choice for many diseases. However, only 1/3 of patients have histocompatible family donors. One approach to this problem is selection of HLA/MLC matched donors from a pool of unrelated individuals. Using the HLA A,B-typed donor files at the BCSEW unrelated donor searches were made for 229 individuals. Donor screening was designed to maximize confidentiality and lack of pressure. HLA-A,B identical donors were found for 176 individuals but only 12/115 of these had one or more HLA-A,B,DR identical MLC-compatible donors. Complete donor searches required 4-12 weeks. Donors ages were 29-49 years. Nine patients (2 ALL, 2 AML, 4 CML, 1 aplastic anemia) aged 2-40 (median 24) years were transplanted using current protocols at the treating institution. Only 2 patients survived (48+ months, aplastic anemia; 6+ months, CML). The longest survivor has severe but improving chronic GvHD. Deaths were due to infection (3), interstitial pneumonitis (2), relapse (1) and GvHD (1). Four of 8 patients at risk had GvHD $\geq$grade II. We conclude that: 1) Unrelated matched marrow donors can be found for only a small percentage of patients. 2) Severe acute/chronic GVHD may occur despite HLA-A,B,DR,MLC matching. 3) Successful donor searches require considerable time. 4) An advantage for matched unrelated donors versus haploidentical family donors has not been shown. The use of matched unrelated donors should be critically evaluated especially in view of recent progress with T-depletion of marrow grafts.

P49 ABNORMAL T CELL RESPONSE TO INTERLEUKIN 2(IL2) IN PATIENTS RECEIVING SOYBEAN AGGLUTININ (SBA) T CELL DEPLETED MARROW, Morton Cowan and Wayne Smith, Dept. of Pediatrics, University of California, School of Medicine, San Francisco, CA 94143.
We evaluated at 2-3 years post bone marrow transplant (BMT) the lymphocyte response to IL2 and phytohemagglutinin (PHA) in 5 patients with severe immunodeficiency disease who received SBA processed T depleted parental marrow. 4/5 were haploidentical (HI) and 1 was a phenotypic match. All had documented lymphocyte engraftment, none had graft vs. host disease, and none received post transplant chemotherapy. We found that in the 4 receiving the HI BMT there appeared to be little proliferative response to IL2 in the presence of PHA until 12 months post BMT. Also, the responses were relatively greater than that for adult controls (cpm, mean ± SD):

	PHA	PHA+IL2	FOLD INCREASE
CONTROLS (5)	21619±12696	44581±8088	2.0±0.9
PATIENTS (5)	10113±7501	33824±19046	5.0±3.2

Phenotyping studies in 3 of the patients were done. While the relative % of fresh cells expressing T3,T4,T8, leu 11, sIg, and Ia were the same as control; there was a notable absence of cells expressing IL2 (0 vs.6±5). Furthermore, when the cells were incubated with PHA and IL2, while there was no difference between patients and controls in T3 and T8 expression there was a marked decrease in T4 positive cells (26±8 vs. 43±11). These data suggest that patients receiving SBA processed, T depleted BMT have subtle but measurable abnormalities in T cell immunity for up to 2-3 years post transplant.

P50 THE EFFECT OF DONOR HETEROGENEITY ON THE SEPARATION OF HUMAN PERIPHERAL BLOOD PROGENITORS AND T LYMPHOCYTES, Douglas C. Dooley, Ping Law, and P. Alsop, American Red Cross, Bethesda, Maryland 20814

If methods were available for depleting donor T lymphocytes, allogeneic transplantation of peripheral blood stem cells might be feasible. Density gradient centrifugation is one approach to the problem. We have determined the reliability and efficacy of the method in a study of 89 random donors. The technique isolated a progenitor enriched population depleted of 92% of T lymphocytes. However, the ability to recover progenitors in the T cell depleted fraction fluctuated widely between donors. To find the source of the variation, the density distributions of T cells, BFUe, CFUc, and low density MNC were determined. Data revealed that progenitor cell density varies widely within the donor pool. The relationship between progenitor and MNC density distributions are donor specific. Donor heterogeneity and overlapping density profiles decrease the reliability of the technique. Those problems cannot be overcome through adjustments in the gradient. Clinical application of the method should be pursued with caution.

P51 IS PROPHYLAXIS AGAINST ACUTE GRAFT VERSUS HOST DISEASE NECESSARY IF TREATMENT IS EFFECTIVE AND SURVIVAL IS NOT IMPAIRED? Gerald J. Elfenbein, Theresa Goedert, John Graham-Pole, Suzanne Skoda-Smith, Samuel Gross and Roy Weiner, Bone Marrow Transplant Program, University of Florida, Gainesville, FL 32610.

There are no compelling data that prophylaxis for acute graft versus host disease (AGVHD) improves survival for patients receiving genotypically HLA identical bone marrow transplants (BMT). We allocated 65 consecutive allogeneic BMT recipients to receive either 3 months of methotrexate (MTX), 3 months of methotrexate plus 4 weeks of prednisone (M+P), or no prophylaxis against AGVHD (NORX). 3 patients who rejected their grafts are excluded.

RX	No.	Age(Range)	AGVHD	IPn	CGVHD	Alive
MTX	28	19(1-36)	7(25%)	7(25%)	16(57%)	9(32%)
M+P	19	14(1-33)	5(26%)	4(21%)	11(58%)	8(42%)
NORX	15	11(1-29)	10(67%)	4(27%)	6(40%)	8(53%)
P(2-tailed)		NSD	<0.015	NSD	NSD	NSD

Subject groups did not differ significantly with respect to age, race, sex disease, donor sex, or sex match. Severe AGVHD (Grade 2-4) was significantly more common for NORX patients. The increased incidence of AGVHD did not result in increased interstitial pneumonia (IPn), increased chronic GVHD (CGVHD) or decreased survival. IV methylprednisolone (5 mg/kg/day) was administered promptly to patients who developed AGVHD. The regimen dictates rapid escalation if patients do not respond quickly, slow tapering for responders and small decrements for tapering. 19/22 (86%) patients responded (95% confidence limits 72% to 100%). All 3 failures were protocol violations. Thus, there was less AGVHD with MTX or M+P than with NORX, but because of successful therapy for AGVHD there is no difference in survival.

P52 THE MODIFICATION OF MURINE GRAFT-VERSUS-HOST DISEASE BY IN VIVO ADMINISTRATION OF ANTI-LFA-1 ANTIBODIES, Loren D. Fast, Denise Davignon and Maurice M. Albala, Rhode Island Hospital and Brown University, Providence, Rhode Island 02902.

A number of monoclonal antibodies capable of inhibiting lymphocyte function have been identified. A murine model of graft-versus-host disease (GVHD) was used to evaluate the therapeutic potential of these inhibitory monoclonal antibodies for the prevention and treatment of GVHD. Initial experiments utilized anti-LFA-1 monoclonal antibodies because they have been shown to inhibit a number of lymphocyte functions including cytolytic function and proliferative responses to alloantigen. Acute GVHD was initiated by intravenous injection of 10^8 C57BL/6 spleen cells into unirradiated (C57BL/6 x DBA/2)F_1 recipient mice while chronic GVHD was initiated by injection of 10^8 DBA/2 spleen cells. Control mice did not receive any cells. On days 0,1,2,3 a subgroup from each group received intraperitoneal injections of 30 µg of rat anti-LFA-1 monoclonal antibody which inhibited lymphocyte function (M17/4.44, IgG2a), 30 µg of rat anti-LFA-1 monoclonal antibody which did not inhibit function (M18/2a, IgG2a) or PBS. Subsequent measurement of parameters associated with GVHD indidated the administration of M17/4 resulted in inhibition of in vivo CTL generation, reversed the suppression of immunoglobulin production, prevented the runting seen in acute GVHD and glomerulonephritis seen in chronic GVHD. Administration of M18/2 did not alter in vivo lymphocyte function but also prevented runting. These findings indicate that administration of anti-LFA-1 antibodies at the initiation of GVHD can modify the course of GVHD. Additional studies to further characterize the in vivo behavior of anti-LFA-1 antibodies are in progress.

P53 EX VIVO TREATMENT OF BONE MARROW INOCULUM WITH IMMUNOTOXIN IT 101 TO PREVENT ACUTE GRAFT VERSUS HOST DISEASE (GVHD) IN ALLOGENEIC BONE MARROW TRANSPLANTATION. Axel A. Fauser, Adrian Langleben and Chaim Shustik, Division of Hematology, Bone Marrow Transplant Unit, Royal Victoria Hospital, McGill University, Montreal, Canada.

Acute GVHD remains a principal cause of morbidity and mortality in allogeneic bone marrow transplantation. GVHD can affect 30-70% of the recipients of transplants from fully matched siblings and may be an indirect cause of death in 20-40% of affected individuals. Certain host characteristics have been associated with an increased risk of GVHD in multiple studies, such as increased host age, clinical status, and nature of the disease. We report our clinical experience of the ex-vivo treatment of allogeneic marrow using a pan-T monoclonal antibody (T101) coupled to Ricin-A-chain. Five patients received allografts from HLA identical and MLR nonreactive sibling donors. All patients had malignant hematological disease with poor prognosis (acute leukemia in second or third remission, 1 non Hodgkin lymphoma stage IV, 1 Hodgkin's disease stage IV, 1 Burkitt's lymphoma stage IV, and 1 acute lymphoblastic leukemia of T8 phenotype). The patients were at high risk for acute GVHD (older than 30 years, and/or subsequent remission). The patients at high risk for acute GVHD received marrow treated ex-vivo with IT-101. The IT-101 treatment removed 97.5± 2.1% of the T lymphocytes. Engraftment was achieved in those patients in 18 days to reach 0.5 x 10 granulocytes/ liter. One patient died at day 11 of fulminant hepatitis with a granulocyte count of 2.5 x 10 per liter. No acute GVHD was observed in this group of patients.

P54 A PHASE I STUDY OF ANTI-T CELL ANTIBODY TREATMENT OF ALLOGENEIC MARROW PRIOR TO TRANSPLANTATION, Joseph W. Fay, Marvin J. Stone and Susan Burkeholder, Sammons Cancer Center, Baylor U. Medical Center, Dallas, Texas 75246

We have transplanted 16 patients (pts) with hematolgic malignancies with intensive chemoradiotherapy and allogeneic marrow that had been incubated in vitro with 3A1 and 16B2 monoclonal antibodies and rabbit complement. These antibodies effectively destroy clonogenic T-cell colonies in vitro. 6/16 pts had HLA mismatched donors (3 mismatched at 1 haplotype). 7 pts were transplanted in remission with acute leukemia or chronic phase CML. 9 pts had advanced leukemia or lymphoma. Median age was 37(0.4-43)yrs. 15/16 pts had good marrow engraftment, 1 pt died of sepsis at day 18. There have been no marrow failures with a median follow up of 5(2-12) months. There has been no significant graft-versus-host disease (GVHD) in 10 HLA identical recipients. Of the 6 mismatched recipients, 1 pt had moderate GVHD, 4 pts had none (1 pt) or mild (3 pts) GVHD, 1 pt died early of sepsis. These data indicate that in vitro treatment of allogeneic marrow with the combination of 3A1, 16B2 monoclonal antibodies and rabbit complement effectively reduce acute GVHD without preventing sustained marrow engraftment.

P55 NATURAL KILLER CELL INVOLVEMENT IN ACUTE GVHD OF THE SKIN
James Ferrara, George Murphy and Steven Burakoff, Dana Farber Cancer Inst and Brighman and Women's Hospital, Boston, MA 02115

We have developed a murine model of acute, lethal GVHD to minor histocompatibility antigens using congenic mouse strains (B10.BR → CBA) which are matched at the Major Histocompatibility Complex ($H\text{-}2^k$). Addition of 10^7 donor splenic T cells to 10^7 donor bone marrow cells produces clinical signs of GVHD including weight loss, diarrhea, hunch posture and rapid mortality (2-4 weeks). Although macroscopic skin changes are minimal, histopathologic evaluation of the organ revealed basal cell vacuolization, exocytosis and satellitosis of mononuclear cells in the epidermis which are strikingly similar to the findings in human disease. The predominant mononuclear cell infiltrate in affected skin hasphenotypic characteristics of natural killer cells. By transmission electron microscopy they had indented nuclei with coarsely clumped heterochromatin and membrane bound cytoplasmic granules and parallel tubular arrays. By immunohistocytochemistry the majority of these mononuclear cells were Thy 1^+, Mac 1^+, AsialoGM_1^+, Ia$^-$, Lyt 1^-, Lyt 2^-. These cells were often found in apposition to necrotic epidermal cells. These data suggest that natural killer cells are found in close approximation to the site of target cell injury and they may play an important role in the pathogenesis of GVHD of the skin.

P56 CYCLOSPORIN A IN THE TREATMENT OF ACUTE GRAFT VS HOST DISEASE, G. Fyles, H.A. Messner, M.D. Minden, J.E. Curtis, G. Lockwood, D. Tritchler, Ontario Cancer Institute, University of Toronto, Toronto, Ontario, Canada , M4X 1K9

A study to assess the efficacy of Cylosporin A (CyA) in the treatment of acute graft vs host disease (GvHD) was carried out from January 1983 to May 1985. During this time 71 patients underwent BMT (CyA group) and were compared to the 73 previous BMT patients (control group). Both groups were similar in age, sex, and conditioning regimen. GvHD prophylaxis in the control group was MTX or MTX and prednisone; acute GvHD in this group was treated with varying doses of prednisone +/- ATG. GvHD prophylaxis in the CyA group was MTX and prednisone. Patients in the CyA group developing GvHD $\geq$ grade 2 received CyA 6.25 mg/kg po q12h; if there was GI involvement CyA 2.5 mg/kg IV q12h over 4 h was given. The dose was adjusted depending on trough CyA levels and serum creatinine. In the control group 45 patients developed GvHD $\geq$ grade 2; 13/45 or 29% survived. In the CyA group 41 patients developed GvHD $\geq$ 2; 22/42 or 54% survived. This was a significant improvement in survival ($p<0.01$) (Wilcoxan). When assessed for age the greatest benefit was for patients greater than age 28; 21/36 (58%) in the CyA group vs 11/34 (32%) in the control group survived ($p<0.02$) (Wilcoxan). CyA was discontinued in 5 patients due to toxicity. We conclude that CyA improved survival in patients with $\geq$ grade 2 GvHD, particularly in the age group over 28 years.

P57 GVHD PREVENTING IMMUNOSUPPRESSIVE REGIMENS: INFLUENCE ON IMMUNE REACTIVITY AFTER IRRADIATION (TBI). W.Gassmann, H.-U.Wottge, M.v.Kolszynski, W.Müller-Ruchholtz, II.Dept.of Internal Medicine and Dept.of Immunology, University of Kiel, D 2300 Kiel (FRG).
In the context of experimental bone marrow transplantation in rats, we have previously demonstrated strong classical immune reactivity persisting after sublethal and lethal irradiation (Transplantation, in press; Exp.Hematol. 13: 430 (1985)abstr.). In a second step we investigated the influence of GVH preventing regimens on this persisting immune reactivity. Methods: rats were irradiated sublethally with 10.5 Gy or lethally with 12.3 Gy and grafted with allogeneic skin. One group of animals was only skin grafted, whereas further groups additionally received irradiated bone marrow of skin donor type i.v. Immunosuppressive regimens tested were (1) 10 mg/kg cyclosporin A/day orally, (2) 0.25 mg/kg methotrexate i.p. on days 1, 3, 6, 11, 18 and 25, and (3) 1 mg /kg prednisolone i.p. daily. Results: (1) As expected the irradiated rats retained the allogeneic skin graft for a median of 28 days. (2) If they received irradiated skin donor bone marrow (10^8 cells) they rejected the skin rapidly. (3) This rapid rejection induced by the skin donor marrow could be prevented by methotrexate (median 19 days), cyclosporin A (median 29 days), but not by prednisolone (median 8 days). Conclusion: These data provide strong evidence suggesting that postgrafting immunosuppression may be of value in facilitating durable engraftment.

P58 HUMAN XENOSPECIFIC T8+ CYTOTOXIC CLONES MEDIATE TISSUE INJURY IN VIVO, Ronald E. Gress, Stephen I. Katz, and Philip J. Lucas, Immunology Branch and Dermatology Branch, NCI, Bethesda, MD 20892
Identification of the human T cell subsets involved in the generation of tissue injury in allospecific T cell responses would provide a basis for selective depletion of those subsets from the marrow inoculum to prevent graft-versus-host disease in allogeneic bone marrow transplantation. In addition, understanding the cellular events leading to such injury might aid the development of therapeutic interventions such as the administration of antibody which blocks those events. A model system was therefore established to allow the study of human effector cells in vivo. We generated H-2^b specific human anti-mouse xenoreactive T cell lines and clones and evaluated the recognition of xenoantigens, the role of cell surface accessory molecules in those cellular interactions, and the ability of these lines and clones to mediate tissue injury in vivo. Human xenoreactive cytotoxic T cells were specific for polymorphic determinants of Class I murine MHC gene products and exhibited fine specificity in distinguishing among targets within a series of K^b mutants. Antibody blocking studies showed certain determinants on T cell accessory molecules to be variably utilized. The intradermal injection of cytotoxic, but not non-cytotoxic, T8+ lines and clones resulted in tissue injury as determined by gross anatomical and microscopic changes (coded biopsies). We conclude that human effector cells variably utilize certain determinants of accessory molecules, that cloned T8+ human effector cells are capable of mediating tissue injury in vivo, and that this ability correlates with cytotoxic activity in vitro.

P59 IMMUNODEFICIENCY IN MICE WITH MINOR ANTIGEN GVHD. BL Hamilton, TR Jackson, G Hastings, S Budhecha, and HD Ochs, Departments of Biological Structure and Pediatrics, University of Washington, Seattle, WA, 98195.
Graft-versus-host disease (GVHD) is associated with a severe immunodeficiency syndrome (GVH-IDS) that develops in the donor derived immune system. The mechanism of GVH-IDS was studied in two H-2 compatible strain combinations in which the donor and recipient strains differed at multiple minor histocompatibility antigens (C57BL/6 and LP/J, both H-2^b, and BALB/c and B10.D2/nSN, both H-2^d). Lethally irradiated recipients were transplanted with a mixture of bone marrow plus graded numbers of spleen cells from the appropriate donor strain. Recipient mice were immunized intravenously with the T dependent neoantigen bacteriophage Ø X 174 to measure an in vivo antigen specific antibody response. Mice with GVHD demonstrated a slightly depressed primary and a significantly depressed secondary antibody response to phage compared to normal or to syngeneically transplanted mice. The secondary response was 95% IgM compared to 95% IgG in the control groups. In vitro proliferative response of Lyt-1^+ T cells and in vitro specific antibody synthesis to Ø X 174 antigen were significantly depressed in mice with GVHD. Suppression was not detected in cell mixing experiments. Plaque forming cell (PFC) responses to the T dependent antigen TNP-Sheep red blood cells (TNP-SRBC) were significantly impaired in all mice with GVHD while PFC responses to the T independent antigen TNP-Brucella abortus (TNP-BA) were impaired only in mice with severe GVHD. These data suggest that GVH-IDS results primarily from a defect in T helper cells in mild GVHD and from a combined defect of T and B cells in severe GVHD.

P60 AN EXPLANATION FOR THE DEVELOPMENT OF HYPERGLOBULINEMIA DESPITE POOR PRIMARY ANTIBODY RESPONSES IN RFM/(T_6xRFM)F_1 CHIMERAS WITH HOST VERSUS GRAFT (HVG) SYNDROME. Richard C. Hard, Jr. and Linda L. Benson, Med. Col. of VA, Richmond, VA 23298

HVG Syndrome is the fatal immunodeficiency syndrome which has been reported in 6 strains of inbred mice following their perinatal inoculation with related F_1 hybrid spleen cells. There is severe depletion of T cells, but the B cell system develops early and becomes hyperplastic. Such mice have poor primary antibody responses but develop hyperglobulinemia. To determine the antibody production of donor and host B cells, RFM perinates were inoculated with spleen cells from (T_6xRFM)F_1 donors, which had or had not been sensitized to horseradish peroxidase (HRP). It was shown that HRP-sensitized F_1 donor B cells engrafted and synthesized 99% of the α-HRP antibody because they were responsive to the allogenic HVG effect and to HRP, while unprimed host and donor cells were not. These data coupled with previous work suggested that virtually all the hyperglobulinemia seen in HVG mice was of F_1 donor origin. The poor primary responses were attributed to the successful transplantation of committed donor B cells, the immaturity of host B cells and lack of helper T cells. Implicit in this work is host acceptance of antigen primed F_1 donor B cells in preference to equally histoincompatible donor T cells and unprimed B cells.

Supported by NIH Grant #AI20208 and monies from the Privat and Carnes Memorial Funds.

P61 Consistent engraftment following haploidentical marrow transplantation associated with increasing total body irradiation(TBI) and decreasing T-cell depletion. Henslee PJ, Macdonald JS, Romond EH, Marshall ME, Doukas MA, Fer MF, Metcalfe M, Greenwood M, Geil J, Maruyama Y, Ash RC, Kryscio RJ and Thompson JS. University of Kentucky, Depts of Peds & Med.

Between 5/83 and 6/85, 28 patients(pts), ranging from 10 mo. to 47 yrs of age(mean, 14.2 yrs), received marrow grafts from related, partially-mismatched donors utilizing monoclonal antibodies(MoAb) as purgative agents for removal of phenotypic T lymphocytes as graft-versus-host disease(GVHD) prophylaxis. 25 pts were treated for underlying hematologic malignancy and 3 for non-malignant disorders. Marrow was donated from 19 parents, 10 siblings and 1 son; the opposite parent donating in 4 second transplant attempts following graft failure. Histocompatible lymphocyte antigen determinants A, B and DR were mismatched in 7 donor/recipient (D/R) pairs at 1 locus, in 10 at 2 loci and in 13 at 3 loci. 21 pts were mismatched at the DR loci. 12 D/R pairs demonstrated bidirectional reactivity in mixed lymphocyte culture; 9 unidirectional reactivity and 9 bidirectional nonreactivity. Graft failure was associated with a preparative regimen including $\leq$ 1200r TBI and T-cell depletion using a MoAb cocktail(T10B9+ T12A10) in 6/10 pts as compared with 15 subsequent pts all demonstrating consistent engraftment following 1400r TBI and marrow purging with a single MoAb(T10B9)($p=0.06$). 1/6 pts with graft failure established a stable graft following 2 marrow boosts from the same donor, resulting in disease free survival(DFS) 785+ days. Although the incidence of GVHD was higher (86.6% vs. 62.5%) following single MoAb-mediated T-cell depletion, 9/13 pts developed mild and manageable GVHD; 7/15 pts with DFS 119+-547+ days. We conclude that haploidentical BMT is feasible following preparative regimens with 1400r TBI and single MoAb T-cell depletion.

P62 SUCCINYLACETONE PREVENTION OF GVHD IN BONE MARROW TRANSPLANTATION, Richard A. Hess, R. Michael Blaese, and Donald P. Tschudy, NCI, NIH, Bethesda, MD 20892

A non-toxic immunosuppressive agent capable of preventing lethal graft versus host disease (GVHD) while permitting normal engraftment during allogeneic bone marrow transplantation is a major goal for clinical transplantation. Succinylacetone (SA) a seven carbon organic ketoacid inhibitor of the second step in heme biosynthesis has previously been shown to profoundly suppress antibody production and inhibit allogeneic tumor graft rejection in rats. In the present studies, we tested SA for its effects on bone marrow engraftment, GVHD, and general toxicity. Lethally irradiated (1000R) adult Wistar Furth rats were reconstituted with 60 x 10^6 syngeneic or allogeneic (Fischer 344) lymphohematopoietic cells consisting of equal numbers of bone marrow and spleen cells. Unreconstituted rats all died following irradiation (12 day median survival). Rats reconstituted with syngeneic cells all survived beyond 220 days and hematologic reconstitution was identical whether or not the animals were given SA (sub q in oil, 800 mg/kg q 3 days x 7 + ip by osmotic minipump 14 mg/day for 2 weeks). In addition, no evidence of renal, cardiac, neuro or hepatotoxicity was observed in the SA treated group. Untreated recipients of allogeneic cells all developed severe GVHD and died (24 day median survival). By contrast recipients of allogeneic cells treated with SA for 26 days post transplantation had a 92% survival at 220 days and were fully chimeric as demonstrated by MHC typing of peripheral lymphocytes. Therefore, SA treatment appears to be effective in preventing lethal GVHD in recipients of allogeneic lymphohematopoietic cells while permitting normal hematopoietic reconstitution in the absence of significant toxicity to other major organ systems.

P63 ALLORESISTANCE TO ENGRAFTMENT OF ALLOGENEIC DONOR BONE MARROW IS MEDIATED BY AN LYT2$^+$ T-CELL IN MIXED ALLOGENEIC RECONSTITUTION, Suzanne T. Ildstad, Jeffrey A. Bluestone, David H. Sachs, National Institutes of Health, Bethesda, MD 20892

We have recently developed a murine model for bone marrow transplantation using mixed syngeneic and allogeneic bone marrow for reconstitution and tolerance induction. The present study attempts to identify critical cell types responsible for alloresistance to engraftment in this model. Lethally irradiated B10 recipient mice reconstituted with T-cell depleted syngeneic (B10) plus T-cell depleted allogeneic (B10.D2) bone marrow repopulated as mixed lymphopoietic chimeras and were specifically tolerant to the B10.D2 allogeneic donor as assessed by skin grafting and by in vitro assays (MLR and CML). If the T-cell population was not depleted from the syngeneic component of the mixed allogeneic bone marrow inoculum, animals repopulated as fully syngeneic and were not tolerant to B10.D2. The syngeneic cell phenotype responsible for this alloresistance to engraftment was studied by selective depletions of various T-cell subsets (L3T4$^+$, Lyt2$^+$, Thy-1$^+$) from the syngeneic component of the mixed bone marrow inoculum using monoclonal antibodies plus complement, and was found to reside among the Lyt2$^+$ subset of T-lymphocytes. To the extent that a similar cell type in the recipient mediates alloresistance in general, targeting of treatment with monoclonal antibodies may provide a specific approach for conditioning of bone marrow transplant recipients.

P64 C1q COATED MICROSPHERES AND MONOCLONAL ANTIBODIES USED FOR THE REMOVAL OF SELECTED POPULATIONS OF ARTIFIC -IALLY "CONTAMINATING" BONE MARROW. J.T.KEMSHEAD*. L.HEATH*. P.LIBERTI$^+$. *ICRF Oncology Lab., Institute of Child Health, London. $^+$ Immunicon Corp., Huntingdon Valley, Pennsylvania.USA.

C1q a component of the complement cascade binds to antibody antigen complexes in the presence of free antibody. This property can be made use of in simplifying procedures involved in the immuno-magnetic separation of cells in bone marrow. Human purified C1q can be physically absorbed onto M450 magnetic microspheres with saturable binding occuring at levels of between 6-10 ug C1q/mg beads.

The experimental systems used involve titrating either human T cells or neuroblastoma cells labelled with the DNA collating Dye Hoerchst 33342 into peripheral blood or bone marrow. Monoclonal antibodies binding selectively to the Hoerchst labelled population are centrifuged to remove aggregates and a panel added to the cells. Directly after this C1q coated beads (approx 100 beads/tumour cell) are added and the mix rotated for 30 mins at 4^{o}C. Depletion of the Hoerchst labelled population can be achieved directly by passing the cells through a magnetic field generated by samarium cobalt magnets, without significant loss of committed haemopoietic progenitor cells (CFUc, BFUe, CFUgemm). C1q coated microspheres have been shown to bind to a variety of monoclonal antibody isotypes once these are bound to antigen, and this appears independent of the reagents ability to activate the complement cascade. The efficiency of the technique will be compared to that of anti-mouse Ig coated microspheres and the results discussed in the context of a rapid double depletion procedure for T cell removal from bone marrow.

P65 PHENOTYPE OF T CELLS CAUSING LETHAL GRAFT-VERSUS-HOST DISEASE IN IRRADIATED MICE, Robert Korngold and Jonathan Sprent, The Wistar Institute, Philadelphia, PA and Scripps Clinic & Research Foundation, La Jolla, CA

The transfer of unprimed T cells to irradiated allogeneic mice expressing differences at either multiple minor histocompatibility (H) or Major Histocompatibility Complex (MHC) loci can result in a high incidence of lethal graft-versus-host disease (GVHD), depending on the dosage of donor cells and the particular immunogenetic barriers involved. In the MHC both H-2 (K/D) class I and class II (Ia) incompatibilities can lead to lethal GVHD, although anti-class II GVHD tends to be more severe. Which particular T cell subsets account for lethal GVHD is not yet clear. In the case of H-2-compatible mice with multiple minor H differences, pretreatment of donor cells with reagents for phenotypic surface markers in the presence of complement have suggested a major role for Lyt-2+ T cells in causing GVHD. Helper-type Ly-1+,Lyt-2- T cells neither seem to be required for GVHD induction nor are capable of causing disease on their own. In the same way, it has been suggested that GVHD directed to either whole MHC or class II differences are caused predominately by L3T4+ T cells, whereas L3T4-, Lyt-2+ T cells cause GVHD to class I (H-2K) differences. Antibody plus complement depletion of donor cells, however, particularly with anti-L3T4 reagents, may leave a residual contamination of undesired cells which can make proper interpretation of data difficult. We have therefore combined this depletion with a positive selection panning technique to obtain pure subset populations. Our current findings employing this procedure on the phenotype of T cells causing lethal GVHD will be discussed.

P66 IMMUNORECONSTITUTION IN SCID AFTER HAPLOIDENTICAL BMT: CORRELATION WITH PRETRANSPLANT FINDINGS, Wilhelm Friedrich, Wolfram Ebell, Shraga S. Goldmann and Bernhard Kubanek, University of Ulm, D-7900 Ulm, FRG

The use of T cell depleted marrow grafts has been effective to prevent GvHD in patients with SCID following HLA haploidentical BMT. However, results regarding immunological reconstitution in these patients have been variable, ranging from graft failure to prompt and complete reconstitution. The basis of this variability currently is poorly understood.
We performed haploidentical BMT in 17 patients with SCID. Distinct correlations were noted between the observed patterns of immune reconstitution and a number of pretransplant findings characterizing defined variants of the disorders. Thus, in six patients with the "low T, normal B cell" variant, prompt complete T cell reconstitutions were observed in five, and reconstitution of B cell functions in four patients. In contrast, in four patients with the "low T, low B cell" variant, this pattern of T cell reconstitution was noted in only one, while in three T cell functions have remained subnormal up to now. In addition, in the latter group no reconstitution of B cell functions was observed.
In the group of patients with incomplete T cell reconstitution, normal NK cell activities were present before BMT, while in the other group these were always absent. In a patient with ADA deficiency as well as in one with the "normal T, normal B cell" variant, complete graft failures were observed. As a consequence, cytoreductive conditioning was used in additional patients with these variants which allowed engraftments and complete reconstitutions. Our experience strongly suggests the need to modify transplant protocols in selected patients with SCID in order to obtain complete immunological reconstitution.

P67 PREDICTIVE VALUE OF URINARY ENZYMES AND TAMM-HORSFALL PROTEIN AS INDICES FOR NEPHROTOXICITY IN PATIENTS TREATED WITH BONE MARROW TRANSPLANTATION, Boris Labar, Dubravka Čvorišček, Vinko Bogdanić, Ana Stavljenić and Franjo Plavšić, Bone Marrow Transplant Centre, Clinical Hospital Rebro, Zagreb, Yugoslavia

Alanine-aminopeptidase (AAP) /EC 3.4.11.2/, N-acetyl-beta-D-glucosaminidase (NAG) /EC 3.1.1.7/, lactate dehydrogenase (LD) /EC 1.1.1.27/, lysozyme (Lys) /EC 3.2.1.17/ and Tamm-Horsfall protein (THP) were determined in urine for monitoring renal function in 21 patients (6 with AML, 5 in first complete remission (CR) and one in relapse, 6 with ALL in first or second CR, 5 with CGL in chronic phase and 4 with SAA) who underwent bone marrow transplantation (BMT).

These enzymes and THP were measured on every second day from 5th day before to at least day 60 after BMT. Lys, AAP and LD were increased while NAG in all patients remained normal. In some patients THP concentrations were found extremely increased.

The correlation and importance of increased enzyme activities and THP concentrations as a sign of nephrotoxicity, especially cyclosporin nephrotoxicity will be presented.

P68 THE ROLE OF BONE MARROW (BM) T CELLS IN IMMUNOLOGICAL RECONSTITUTION OF ALLOGENEIC BM CHIMERAS. Leshem, B., Tsuberi, B., Lebendiker, Z., Weiss, L., Slavin, S. and Kedar, E. The Lautenberg Ctr. and the BM Transplantation Unit, Hebrew Univ.-Hadassah Med. School, Jerusalem, Israel.

Graft vs. host disease can be prevented by in vitro depletion of T-cells from BM prior to BM transplantation (BMT). We assessed the role of BM T-cells in the reconstitution of various immune functions following allogeneic BMT. Lethally irradiated CBA/J ($H\text{-}2^k$) mice were infused with 10^7 unseparated (WBM) or T-cell depleted (TDBM) B10.BR($H\text{-}2^k$, minor H-2 disparate) BM cells. Various immune functions of spleen cells (SC) of chimeras were tested 2-12 wks post BMT and compared with those of normal donors. We demonstrated that: (a) T-cell depletion of BM prior to BMT did not significantly alter the rate of reconstitution of immunological functions. (b) BM colonies on soft agar and proliferative response to LPS were restored at 4-8 wks post BMT. (c) Reconstitution of allocytotoxicity (CML) and proliferative response to allogeneic leukocytes (MLR), ConA and PHA did not exceed 50% even at 12 wks. (d) At 4 wks post BMT, SC from WBM and TDBM chimeras suppressed the alloreactivity (MLR and CML) of SC of normal CBA/J while only SC from WBM, but not from TDBM chimeras, suppressed the alloreactivity of normal B10.BR SC. SC of neither WBM nor TDBM chimeras suppressed the MLR and CML of unrelated C3H SC. (e) At 2 wks post BMT, non specific suppression of alloreactivity was exerted by SC of both BM chimeras, while at 10 wks, they partially suppressed only CBA/J SC. (f) The restricted suppression was mediated by $Thy1.2^+$ cells. The potential role of the suppression is currently under investigation.

P69 CADAVERIC BONE MARROW HARVEST FOR USE IN ALLOGENEIC HUMAN MARROW TRANSPLANTATION, Philip J. Lucas, Ralph R. Quinones and Ronald E. Gress, Immunology Branch, NCI, Bethesda, MD, 20892

Elimination of T lymphocytes from donor bone marrow (BM) has been shown to significantly reduce the incidence of graft-versus-host disease (GVHD) in allogeneic marrow transplantation. In animal models the extent of depletion is a critical factor in protection from GVHD with the presence of as few as 0.1% T cells correlating with the development of GVHD. We have therefore evaluated whether the method of BM harvest can influence the extent of that depletion in man. Conventionally aspirated iliac crest and surgically resected rib marrows were treated by SRBC rosetting and a pool of four monoclonal antibodies (MAb) -- CD2, CD5, CD7 and unassigned (but pan T cell) specificities -- plus rabbit complement. After such depletion, aspirated BM had no detectable T cells by FACS analysis and MLR/mitogen proliferation assays, but contained between 0.1-1.0% T cells as determined by a limiting dilution assay (LDA). Surgically resected marrow after depletion had <0.001% residual T cells by LDA. These results were then applied to cadaveric vertebral bodies (VB) containing clinically relevant quantities of marrow. Nine separate VB marrow harvests yielded an average of $5.2 \times 10^9 \pm 1.4$ mononuclear cells/VB with 4-5 VB harvested per procedure. Large scale depletion of 5 VB resulted in a final yield of 9.7×10^9 mononuclear cell (44.1%) with < 1 T cell/10^5 marrow cells by LDA. We conclude that the method of marrow harvest can influence the final extent of T cell depletion, that it is possible to obtain clinically useful quantities of marrow by surgical resection, and that the LDA determines T cell content at the low levels shown to produce GVHD in murine models.

P70 CRYOPRESERVED HUMAN CADAVERIC BONE MARROW: POSSIBLE ALTERNATIVE SOURCE OF TRANSPLANTABLE MARROW. A.Melaragno, J.Maples, C.P.Reynolds, D.Vembu, and S.Lewis, Naval Med.Res.Inst.,Bethesda,MD. We have developed methods to procure,process, and freeze bone marrow(BM) from human cadaveric vertebral bodies(VB). VB are ground mechanically, filtered through mesh screens, and incubated with DNAse for 2 hours prior to freezing in a fetal calf/DMSO solution. We have processed VB from over 200 donors. The average yield of BM cells is 2.1x10E10/donor and viability is > than 85%. Over 50% of the BM cells are recovered post freezing with a viability in excess of 80%. BM cells from VB grow in colony-forming assays and long-term liquid culture systems. Thus,sufficient viable BM cells can be harvested from a single cadaveric donor for a BM transplant. Clinical studies testing the efficacy of cadaveric BM are warranted. NMR&DC M0095.001.0003.

P71 A ROLE FOR LYT-1^+ CELLS IN MOUSE MINOR HISTOCOMPATIBILITY GRAFT-VS-HOST REACTION, James P. OKunewick, Mary J. Buffo, Debbie L. Kociban, Allegheny-Singer Research Institute, Allegheny General Hospital, Pittsburgh, PA 15212.

The role of Lyt-1^+ T-lymphocytes in major histocompatibility GvHR (MHC-GvHR) has been firmly established through monoclonal antibody (MAB) techniques. In similar studies involving a CBA → B10.BR mouse model the minor histocompatibility GvHR (MiHL-GvHR) which developed was shown to be primarily influenced by Lyt-2^+ T-cells. Based on these studies a theory was proposed that MiHL-GvHR is a Lyt-2^+ T-cell dependent class-I immune response, while MHC-GvHR is a class-II response involving Lyt-1^+ cells. However, research in other laboratories has suggested that there may be more than one level of effector cell for GvHR and that, in addition to mature Lyt-1^-2^+ cells, immature Lyt-1^+2^+ donor T-cell progenitors may also play an important role in MiHL-GvHR. Therefore, we have reexamined the question of Lyt-1^+ cells in MiHL-GvHR using two transplant combinations different from those previously employed. These are B10.S →SJL/J and B10.D2 → DBA/2J. The results obtained with both models indicated that, while anti-Lyt-2.2 MAB treatment of the donor cells partially suppressed MiHL-GvHR, treatment with anti-Lyt-1.2 was significantly more effective, with the suggestion that more than one level of effector cell may be involved. In addition, the data obtained from the B10.D2 → DBA/2J transplants suggest that if the donor and recipients are mismatched in T-cell subtypes (Lyt-x.1 vs Lyt-x.2) simultaneous donor cell exposure to both MABs may adversely affect some sort of suppressor mechanism, resulting in graft failure. In light of the above it is suggested that a modification of the previous theory for MiHL-GvHR may be warranted.

P72 HLA-MATCHED T-CELL DEPLETED ALLOGENEIC BMT IN THE TREATMENT OF LEUKAEMIA.

Prentice, H.G., Brenner, M.K., Grob, J-P. Janossy, G., Gilmore, M., Ivory, K., Skeggs, D., Hoffbrand, A.V. Royal Free Hospital, London.

Fifty-six patients (32M, 24F, age range 2.5-46, median 24) with leukaemia (15 AML, 27 ALL, 14 CGL) at various stages of their disease (1CR = 24, 2CR = 10, rel = 8, 1CP = 10, 2CP = 1, ACCEL = 2, BT = 1) have received BMT from HLA-matched sibling donors, between 2/83 and 11/85. Conditioning for BMT consisted of Cy 60mg/kg x 2 and 7.5Gy single fraction TBI (n=39) or recently 8Gy (n=6) or Ara-C 3g/m²x6, Cy 45mg/kgx2 and 7.5Gy (n=8) or 8Gy (n=3). Depletion of T-lymphocytes from donor marrow in vitro was achieved with a cocktail of 2 murine McAbs (MBG6+RFT8, n=43 or RFT12+RFT8, n=13)plus rabbit C mediated lysis. No GvHD prophylaxis was given. Sustained engraftment occurred in 46 of 50 evaluable patients (>60d). Of 54 evaluable for aGvHD, 10 had Grade I, 1 Grade II (age 41) and 1 Grade III (age 31). Four of 34 (12%) evaluable (>150d) developed cGvHD. Twenty-seven deaths have occurred: 16 relapses (d90-401, median d 193), 4 rejections, 4 pneumonitis (2 CMV),2 CCF and one Budd Chiari syndrome. From 34 patients transplanted in 1CR or 1CP, 24 are alive 14-912 days after BMT (median 440d) and one single leukaemia relapse occurred in a patient with CGL (BT without preceding CR). The actuarial DFS is 65% at 30 mo.for this group compared to 7% at 24 mo. in patients transplanted later. BMT using T-cell depletion is safe, an increased risk of relapse is encountered only in patients transplanted at late stages of their disease. These latter patients might benefit from more intensive conditioning.

P73 EFFICACY OF DEPLETION OF T CELLS BY MONOCLONAL ANTIBODIES (MAb) + COMPLEMENT (C'): COMPARISON OF 4 DIFFERENT MAb BY A LIMITING DILUTION T CELL ASSAY, Ralph R Quinones, Jody L Cassell, Philip J Lucas, Ronald E Gress, Immunology Branch NCI, Bethesda, MD,20892

In vitro depletion of T cells from marrow is being utilized to prevent graft-versus-host disease (GVHD) in both matched and mismatched allogeneic bone marrow transplantation (BMT). One approach is the utilization of murine MAb specific for cell surface antigens on human T cells + C'. To optimize depletion, a determination of the efficiency of T cell purging by individual MAb, as well as a mixture, has been performed. These determinations required a T cell assay of adequate sensitivity to quantify very low levels of residual T cells. We therefore compared depletion of peripheral blood T cells by four C'-fixing, anti-human-T-cell MAb in a limiting dilution assay (LDA) for residual T cells, which can detect a 5-6 log decrease in T cells. Peripheral blood mononuclear cells were incubated with MAb, washed, treated with 2 cycles of C', washed, and placed in a LDA to quantify remaining T cell contamination, with results as shown in the table. The mixture of the 4 MAb was then tested on surgically resected marrow and resulted in depletion to < 1 T cell in 10^5 marrow cells. We conclude that this LDA will be useful in optimizing approaches of T cell depletion to levels relevant for GVHD prevention. Using this assay, the mixture of 4 MAb appeared preferable to any single MAb because of greater reproducibility in effective T cell depletion from donor to donor.

MAb	Distribution	CD	Antigen	Subclass	Log Depletion	N
95-5-49	pan T	2	50 KD	IgG2b	2.8	7
95-6-22	pan mature T	-	43-45 KD	IgG2a	1.6	6
T101	pan mature T	5	65 KD	IgG2a	1.9	4
3A1	T subset	7	41 KD	IgG1	0.7	5
Mix of all four		-	--	--	3.5	10

P74 FLOW CYTOMETRIC AND SORTING ANALYSIS OF SOYBEAN AGGLUTININ REACTIVITIES WITH NORMAL ELEMENTS OF PERIPHERAL BLOOD AND BONE MARROW, Christopher L. Reading and Kevin J. Cockerill, M.D. Anderson Hospital and Tumor Institute, Houston, TX 77030

We have analyzed human peripheral blood and bone marrow cells for reactivity with FITC-Soybean Agglutinin (F-SBA) to explore the potential for T-cell removal in allogeneic bone marrow transplantation. Peripheral blood buffy coat cells purified into granulocytes, mononuclear cells, glass wool nonadherent cells, and E-rosette-positive and -negative cells. Bone marrow mononuclear cells were purified from buffy coats on discontinuous Percoll gradients to yield a fraction enriched (10X) in clonogenic hematopoietic progenitor cells with nearly 100% recovery of progenitors. The bone marrow mononuclear cells were analyzed by forward and low-angle light scatter regions for fluorescence after staining with FITC-SBA. Cells were sorted on the basis of fluorescence into positive, negative and "all" fractions, and analyzed in hematopoietic colony assays in methylcellulose. We found that T lymphocytes are unreactive with SBA, and that myeloid blasts are positive. This result was confirmed by lectin peroxidase staining followed by hematoxylin counter staining. Colony assays of sorted bone marrow cells revealed that GM-CFC, BFU-E and GEMM-CFC were distributed between the SBA-positive and SBA-negative cell fractions. We conclude that SBA is highly reactive with myeloid elements in bone marrow cells but has minimal or no reactivity with T lymphocytes. Since SBA agglutination has been used to remove graft-versus-host reactive cells (currently believed to be T lymphocytes) and to enrich for myeloid progenitors in the unagglutinated fraction, we must question the mechanism of this separation. Our results indicate that based on binding, SBA cannot be used to remove T lymphocytes.

P75 IMMUNOTOXIN-MEDIATED T CELL DEPLETION OF BONE MARROW ASSESSED BY LIMITING DILUTION CULTURES. Marta K. Rozans, Brian R. Smith, and Richard A. Miller, Boston, Mass 02118.

Removal of mature T cells from bone marrow has been proposed as a means of preventing graft-versus-host disease following marrow transplantation. In order to determine the clinical usefulness of any depletion method, however, adequate assay systems must be devised to assess the degree of T cell depletion. We have applied 3 sensitive limiting dilution (LDA) techniques to depletion protocols involving marrow treated with anti-Leu-1 (CD5) + C' or ricin A-T101 (CD5) immunotoxin. LDA demonstrated that following two cycles of anti-Leu-1 + C', depletion of cells able to proliferate in response to PHA and interleukin-2 (IL-2) (pPTL) was routinely greater than 99.0% complete, and 96% to 98% of T cells that secrete IL-2 (pHTL) were removed. In comparison, fluorescent cell-sorting analysis (FACS) indicated that 93% to 95.5% of Leu-4^+ cells had been deleted. When the non-cytolytic immunotoxin was used, 97% to 99.9% depletion was seen for pPTL and 86% to 99.9% depletion was noted for pHTL, whereas only 48% to 96% Leu-4^+ cells were removed. We conclude that LDA for functionally intact T cells is more useful than FACS in determining small numbers of residual T cell contamination in marrow after depletion protocols, especially if non-cytolytic procedures are involved.

P76 SWINE GVHD MODEL AND THE EFFECT OF T CELL DEPLETION OF MARROW BY MONOCLONAL ANTIBODIES (T4+T8+T11) PLUS COMPLEMENT, Kaoru Sakamoto, Larry R. Pennington, Frederique B. Popitz, Mark D. Pescovitz, Ronald E. Gress, Shinji Shimada, Stephen I. Katz, and David H. Sachs, National Cancer Institute, NIH, Bethesda, MD 20892

As part of our bone marrow transplantation program in miniature swine, we have established a model of Graft versus Host (GVH) Disease and have examined the effect of T cell depletion of donor bone marrow on prevention of this disease. Using our Major Histocompatibility Complex (MHC) inbred miniature swine, the bone marrow from MHC homozygous ("Parental") swine was injected into irradiated (900R total body irradiation) MHC heterozygous (F1") swine sharing one haplotype. All 16 recipients engrafted and developed skin rashes of varying intensity depending on the level of T cells in the donor inoculum. Of 5 animals receiving untreated bone marrow, 3 showed moderate and 2 showed severe skin rash with histopathological findings similar to human GVHD skin rash. Two animals receiving bone marrow plus additional donor PBL as a source of mature T cells developed the most severe skin rash of the 16 cases. T cell depletion of donor marrow in vitro using a combination of 2 mouse anti-pig T cell antibodies (T4+T8 equivalents) plus complement was found to limit the disease to "moderate" in all four animals tested. A combination of three monoclonal antibodies (T4+T8+T11 equivalents) plus complement decreased the skin rash to the "mild" category in all five animals tested. These results indicate that: 1) this model is useful for the study of GVHD and its prevention and 2) T cell depletion of donor marrow by monoclonal antibodies plus complement is an effective way to minimize GVHD.

P77 KINETICS OF IN VITRO DEOXYADENOSINE (dAdo) TOXICITY FOR PERIPHERAL BLOOD MONONUCLEAR CELLS (PBM) : POTENTIAL T-CELL PURGING STRATEGY, William P. Sheridan and David S. Gordon, Emory University, Atlanta GA 30322

T lymphocytes are sensitive to toxic effects of deoxyadenosine (dAdo) in the presence of inhibition of adenosine deaminase by deoxycoformycin (dCf). Accumulation of dATP leads to NAD depletion and delayed death of resting lymphocytes, and inhibition of RNA synthesis in newly activated lymphocytes which are most sensitive to dAdo in the first 24hr after activation. We studied the proliferative response of PBM to allogeneic PBM (MLR) and to the mitogens phytohemagglutinin (PHA) and monoclonal anti-CD3 antibody OKT3, after incubation with dAdo + dCf for up to 24hr before or after stimulation and with the drugs continuously present. PBM were treated with $5x10^{-5}$M dCf for 60' prior to the addition of dAdo, then incubated at $1x10^6$/ml, 37°C, in Opti-MEM with 20% FCS for 6,12,18 or 24hr, washed x3 and suspended in fresh media. ^{3}H-thymidine was added to 0.2ml aliquots cultured in U-bottom microtitre wells at 48hr for mitogens and 5d for MLR, and the cells harvested 24hr later. Significant inhibition was seen after 18hr preincubation for the PHA response. Incubation during stimulation increased the degree of inhibition achieved. Similar levels of inhibition were seen after 6-12hr incubation for the OKT3 and MLR responses. Incubation with $5x10^{-5}$M dCf and $5-20x10^{-4}$M dAdo for 24hr after stimulation achieved more than 99.9% suppression of the responses to OKT3 and allogeneic PBM. Chemical T cell purging with dCf and dAdo is feasible as bone marrow colony-forming cells are less sensitive to deoxynucleoside toxicity. This T cell purging strategy also has advantages of simplicity and widespread applicability.

P78 ACUTE GVHD AFTER TRANSPLANTATION OF OKT8 POSITIVE CELL DEPLETED MARROW FROM AN HLA MISMATCHED RELATED DONOR, Shintaro Shiobara, Mine Harada, Tamostu Matsuda and Kanazawa University BMT Team, Kanazawa 920, JAPAN

A 9-yr-old male with acute lymphoblastic leukemia developed relapse shortly after the cessation of 3 year's maintenance therapy. He achieved 2nd complete remission and bone marrow transplantation(BMT) was considered. Since he had no sibling, his mother was selected as a donor based on HLA typing. She was mismatched with the patient at HLA-A,B loci on an unshared haplotype. MLC was mutually nonreactive. For mismatched BMT, he was conditioned with high-dose cytosine arabinoside and cyclophosphamide and total body irradiation. In addition intrathecal methotrexate was given twice before BMT. Bone marrow cells from his mother were treated in vitro with OKT8 monoclonal antibody plus complement. The marrow dose was 3×10^7 /kg and OKT8 positive cells were almost completely eliminated. The posttranpslant clinical course was complicated with grade 2 acute GVHD, delayed hematologic recovery and viral encephalitis. Skin rash developed on day 8 and serum levels of totalbilirubin exceeded 2.0 mg /dl on day 28. Biopside specimens of the skin on day 18 were highly compatible with GVHD. Furthermore, majority of mononuclear cells infiltrating the dermis were OKT8 positive by an immune-peroxidase stainging. On day 36 he suddenly lost consicousness with convulsions and died. Postmortem examination revealed partial hemopoiesis in the bone marrow tissue. These observations indicate that in vitro purging of OKT8 positive cells from the marrow inoculum is not effective to prevent acute GVHD in mismatched BMT. Modifications will be required for the establishment of BMT from an HLA mismatched donor.

P79 THE USE OF CAMPATH-1 FOR PREVENTION OF GRAFT VS HOST DISEASE (GVHD) AND TOTAL LYMPHOID IRRADIATION (TLI) FOR ABROGATION OF HOST RESISTANCE TO T-CELL DEPLETED ALLOGRAFTS

S Slavin, G Cividalli, C Brautbar, G Hale, H Waldmann, R Or. Dept of Bone Marrow Transplantation & Immunobiology Research, Hadassah University Hospital, Jerusalem, Israel.

Acute and chronic GVHD were totally prevented in 43 consecutive cases of genotypically matched bone marrow allografts without post-transplant immunosuppression by pre-transplant in vitro depletion of mature T lymphocytes from donor's marrow. Thirty patients underwent allogeneic bone marrow transplantation (BMT) following depletion of donor's marrow with monoclonal rat anti-human lymphocyte antibody (CAMPATH-1) and autologous serum as source of complement. Additional patients with leukemia underwent BMT with T cell depletion by sheep red blood cells with/our soybean agglutinin. Late graft rejection occurred in 5/17 leukemia patients conditioned by cytoxan (CY) 120 mg/Kg and TBI 1200 rad. None of 13 patients conditioned by TLI 150 rad x 4, CY and TBI rejected his graft. Durable engraftment of T-depleted marrow was also achieved in 3/3 severe aplastic anemia patients conditioned with TLI 1800 rad and CY 200 mg/Kg and in 4/4 thalassemia patients conditioned with TLI 800 rad, busulfan 16 mg/Kg and CY 200 mg/Kg. We conclude that GVHD can be totally prevented by depleting T cells pre-transplantation using CAMPATH-1 and autologous serum without post-transplant anti-GVHD prophylaxis. Rejection of T-cell depleted allografts can be prevented by TLI.

P80 PROLIFERATIVE RESPONSES TO HUMAN RIL-2 FOLLOWING MARROW TRANSPLANTATION (BMT)

Paul M. Sondel, Jacquelyn A. Hank Bridget Flynn, Richard Hong, and Peter C. Kohler, University of Wisconsin, Madison, Wisconsin 53792

Proliferative T cell responses to mitogens, soluble antigens, and allogeneic cells remain depressed following BMT long after hematopoietic engraftment. Many months are required for return of normal T cell proliferative responses. Preliminary results suggest the recipients of T cell depleted BMT may have even more severe T cell function defects. Despite these poor T cell responses following BMT, strong T cell proliferation can be induced in vitro by human recombinant IL-2 (kindly provided by CETUS, Emeryville, CA). Lymphocytes obtained from BMT chimeras showed dramatic ^{3}H-TdR incorporation following 5 days of culture with rIL-2, but not following stimulation with mitogens, soluble antigens, or in MLC. These responses to rIL-2 often exceeded parallel responses to rIL-2 or specific antigens or mitogens by lymphocytes from healthy controls.

Eight patients have been studied sequentially; 2 T cell depleted BMT, and 6 receiving whole marrow BMT. All showed the same dramatic proliferative response to rIL-2 as early as 20 days post BMT, as well as at multiple subsequent timepoints.

Whether these IL-2 responsive lymphocytes are alloreactive T cells that express IL-2 receptors due to activation in vivo, primitive regenerating lymphocytes that may be increased in the circulation during immune reconstitution following BMT, or lymphokine activated killer (LAK) precursors activated during immune engraftment, is currently under investigation.

P81 A SUBLETHAL CONDITIONING WHICH INDUCES FULL CHIMAERISM AND PREVENTS SECONDARY DISEASE, Stefan Thierfelder, Udo Kummer, Rolf Schuh and Gertrud Hoffmann-Fezer, Institut für Hämatologie, GSF, Munich, FRG

In vitro T cell depletion of HL-A mismatched bone marrow by monoclonal antibodies (moab) has so far been complicated by graft rejection or residual GVHD. In mice, addition of complement was shown to clearly enhance the antibody effect. However, injection of complement fixing moabs with pan T specificity (anti-Thy-1) usually fails to reduce graft-versus-host or host-versus-graft reactions. We recently reported on anti-Thy-1 moabs which do not need complement to be added in vitro in order to suppress GVHD in vivo. Such antibodies have a high affinity for C1q, the first subunit of the complement cascade. They were found to prevent secondary disease also when injected into irradiated prospective marrow recipients. We now show that a single injection of C1q affine rat or mouse anti-Thy-1 moabs after irradiation and before transplantation suppresses both graft-versus-host and residual host-versus-graft reactions.
This type of conditioning also allows to reduce the dose of total body irradiation down to 5 or 6 Gy for induction of full chimaerism and prevention of mortality from secondary disease following transplantation between homozygous H-2, IA mismatched mice.

P82 EFFECTS OF TOTAL BODY IRRADIATION (TBI) QUALITY ON ALLOGENEIC BONE MARROW TRANSPLANTATION, Huib M. Vriesendorp*, Rod Monroy**, Patricia M. Johnson***, and Thomas J. MacVittie. *Johns Hopkins Oncology Center, Baltimore, MD., **AFFRI, Bethesda, MD., ***Northwestern University, Chicago, IL.

Ionization chambers, TLD's and activation foils were used for dosimetry in dog phantoms and in vivo. Three hundred KV, Cobalt 60, 10 MV photons and 0.8 MeV neutrons were investigated. Neutrons led to non uniform TBI, photons were moderately uniform. Ratio for max/min dose were 1.25 for 300 KV and 1.10 for Cobalt and 10 MV. DLA identical bone marrow cells ($2\text{-}4x10^8$ Kg^{-1}) were given I.V. within 24 hrs after TBI. After maximum tolerated neutron TBI, allogeneic DLA identical marrow was only temporarily accepted. The therapeutic ratio of neutron TBI is negatively influenced by severe GI toxicity. Additional immunosuppression will be required for permanent allogeneic engraftment in patients after an accidental lethal dose of neutron TBI. For photon TBI the incidence of HVG decreased with dose while the incidence of GVH increased with dose. Cobalt and 10 MV TBI gave similar results. The RBE for HVG and acute side effects was approx. 0.9 while for GVH and late side effects the RBE was approx. 1.2. Radiation quality appears to have a profound influence on the effects of TBI. There is an optimal TBI dose with a low HVG and GVH incidence and 300 KV TBI has the highest therapeutic ratio. The influence of TBI dose inhomogeneity deserves to be analyzed further. Deliberate shielding of or boosts to specific volumes could improve upon the low therapeutic ratio of TBI of any quality as a conditioning regimen for allogeneic bone marrow transplantation.

P83 RAT MONOCLONAL ANTIBODIES TO PREVENT G vs H AND H vs G REACTIONS IN BONE MARROW TRANSPLANTATION. H. Waldmann, G. Hale, S. Cobbold, M. Clark, H. Tighe and C. Bindon, University of Cambridge, Department of Pathology, Division of Immunology, Cambridge, England.

We will present data to show that rat monoclonal antibodies may be used to permit fully histoincompatible bone marrow grafts to be achieved in mice by eliminating in vivo, T cells relevant to G vs H and H vs G reactions.

We are trying to evolve suitable cocktails (the CAMPATH series) to minimise such reactions in human marrow transplantation. Information will be provided showing i) the data accumulated from many collaborating centres for more than 200 transplants using CAMPATH-1 and donor complement to remove T cells from marrow; and ii) attempts to construct a suitable cocktail for in vivo use involving anti-T-cell antibodies selected/combined on the basis of their ability to exploit natural effector systems (such as complement lysis) as well as to block function.

P84 TRANSPLANTATION OF T AND B LYMPHOCYTE DEPLETED MARROW FROM MOTHER IN A CASE OF SCID
Yamagami M., Matsuda A., Koizumi S., Taniguchi N., Shiobara S., Harada M., Departments of Pediatrics and Medicine, Kanazawa University, Kanazawa 920, JAPAN

Severe combined immunodeficiency(SCID) is now correctable by bone marrow transplantation. Even if an HLA matched donor is not available, several groups were succeeded in the correction of SCID by treating haplomismatched bone marrow with soybean lectin and E-rosetting or anti-T cell monoclonal antibodies and complement to prevent GVHD. Recently a 1-mo-old body was referred to us for oral thrush. He was diagnosed SCID due to ADA deficiency based on the findings of marked lymphocytopenia(100/μl) and no detectable ADA activity. In an attempt to correct immunodeficiency in this patient, we carried out bone marrow transplantation from his mother. Since she was one haplotype mismatched, bone marrow inoculum was treated in vitro with monoclonal antibodies and complement to eliminate both T and B lymphocytes. We used a cocktail of CT-2,BA-1,BA-2 and BA-3 plus baby rabbit complement to deplete both T and B cells because some cases were reported to develope B cell proliferative disorders, which were presumable related to EB virus-infected B cells from a donor. He was infused with in vitro treated bone marrow cells at a dose of 4.8×10^7/kg. Standard MTX was given for GVHD prophylaxis. At the time of this report (6wk posttransplant), peripheral blood lymphocytes recovered up to 300/μl. They were $OKT3^+$ (35%),$OKT4^+$ (20%) and $OKT8^+$ (15%). However, PHA responsiveness has not yet been demonstrated. The patient is now alive and well in the absence of acute GVHD. Second transplant is considered for the complete correction of SCID in this patient.

P85 CURRENT STATUS OF BONE MARROW TRANSPLANTATION IN LEUKEMIA USING DONORS OTHER THAN HLA-IDENTICAL SIBLINGS, Ferry E. Zwaan, Department of Hematology, University of Leiden, the Netherlands.

For the advisory committee of the International Bone Marrow Transplant Registry (IBMTR). In the data base of the IBMTR information is available on a total of 380 patients, who underwent bone marrow transplantation with donors other than HLA-identical siblings. The number of patients with the disease categories AML, ALL, and CML, who were transplanted in 1st remission (or chronic phase) was 98. The results of these transplants were compared with 916 transplants performed with HLA-identical donors. Patients transplanted with class I or class II antigens mismatched family donors, demonstrated a 3-year actuarial survival of 44% and 47%, respectively. Although these results are significantly different ($p < 0.008$) compared with transplants with HLA-identical siblings (3-year survival: 49%), they are promising. There was no significant anti-GVHD effect of T-cell depletion in these mismatched transplants, whereas a significant difference ($p < 0.001$) was found in the HLA-identical sibling group. No difference was found in relapse rate.
These results are encouraging for further use of mismatched family donors in bone marrow transplantation for leukemia.

Bone Marrow Transplantation

P86 AN AUTOLOGOUS BONE MARROW TRANSPLANTATION/GENE TRANSFER PROTOCOL IN NON-HUMAN PRIMATES USING RETROVIRAL VECTORS, W. French Anderson[1], Philip Kantoff[1], Martin Eglitis[1], Jeanne McLachlin[1], Evelyn Karson[1], James Zwiebel[1], Arthur Nienhuis[1], Stefan Karlsson[1], Patricia Turner[1], Richard O'Reilly[2], Alan Gillio[2], R. Michael Blaese[1], Donald Kohn[1], and Eli Gilboa[3]. [1]National Institutes of Health, Bethesda, MD 20892, [2]Memorial Sloan-Kettering Cancer Center, NY, NY 10021, [3]Princeton University, Princeton, NJ 08544

The correction of an inborn error of metabolism by the insertion of a functioning gene into patients' defective cells is a potential mode of therapy which is now approaching realization. A likely initial disease candidate for human gene therapy is adenosine deaminase (ADA) deficiency, a cause of severe combined immunodeficiency (SCID). A retroviral vector, called SAX, containing the human cDNA for ADA as well as a selectable gene, Neo^R phosphotransferase, has been constructed and shown to be effective in correcting the metabolic defect in ADA(-) cell lines treated in vitro. Using an autologous bone marrow transplant (BMT) protocol with non-human primates, the ADA gene was introduced into bone marrow (BM) cells via retroviral infection with the SAX vector. The donor animals were then lethally irradiated and their gene-treated cells reinfused. Peripheral blood and BM cells were analyzed 3-6 weeks later for the presence of SAX vector DNA and human ADA activity. Approximately 0.1-1% of the BM cells contained vector DNA which restriction digests showed to be intact. Human ADA and Neo^R phosphotransferase were each expressed at low levels. Both monkey and human BM cells are being studied by the CFU-GM assay using the SAX vector in order to determine optimal conditions for retroviral vector infection. Our results suggest that this autologous BMT/gene transfer protocol may be applicable as a model for human gene therapy.

P87 PRE AND POST BONE MARROW TRANSPLANTATION LYMPHOID CELL SUBSET CHARACTERISTICS OF ß MAJOR THALASSEMIC PATIENTS.
Andreani M., De Biagi M.; Centis F.; Rossi C.; Polchi P.; Lucarelli G.
DIVISIONE EMATOLOGICA DI MURAGLIA - OSPEDALE DI PESARO - PESARO - ITALIA.

Cell surface markers and mitogen and allogeneic antigen response of peripheral lymphoid cells were determined in 62 patients affected by ß major thalassemia who received an HLA identical bone marrow transplantation. The study included a pre-transplant and 50-90, 140-190, 340-370 and over 400 days post-transplant investigation in order to evaluate the influence of pre-transplant transfusive treatment and of the GVHD and viral infections in the post-transplant period. No significant difference in the distribution and function of the lymphoid cell subsets was observed in the pre-transplanted thalassemic patients compared to a group of untransfused healthy controls exept for a slight increase of the OKT8 positive cells. The reconstitution of the immunological system after high dose busulfan and cyclophosphamide did not differ from that observed in the group of patients transplanted for other hematological diseases and conditioned with TBI and cyclophosphamide.

Supported by Regione Marche and the Berloni Foundation Against Thalassemia.

P88 ALLOGENEIC MARROW TRANSPLANTATION FOR LEUKEMIC PATIENTS WHO LACK MATCHED SIBLING DONORS, UTILIZING PARTIALLY MATCHED RELATED DONORS IN CONCERT WITH T-CELL DEPLETION FOR GVHD PROPHYLAXIS, R.C. Ash, J. Casper, M.S. Serwint, C. Coffey, J.E. Bruckman, M. Greenwood, J. Geil, E. Romond, B. Camitta, J. McDonald, J. Thompson, and Y. Maruyama, Medical College of Wisconsin, Milwaukee, & University of Kentucky, Lexington

Over 60% of leukemic patients who might benefit from marrow transplantation will not have a matched sibling donor. The use of partially-matched related donor marrow grafts in concert with T-cell depletion for GVHD prophylaxis has been investigated in several centers, but early clinical trials have encountered such limiting problems as marrow graft failure and the appearance of B-cell neoplasia. Over a 32 month period 28 high-risk leukemic patients (age 15 months-32 years, median age 12 years) with advanced neoplastic disease were treated by high dose chemoradiotherapy and partially-matched marrow transplant. Of the first 9 patients treated, 3 died of problems related to graft failure. Subsequent increases in the intensity of pretransplant chemoradiotherapy resulted in sustained marrow engraftment for each of the next 19 consecutive patients. The impact of a treatment schedule which achieves sustained marrow engraftment is manifest: only 1 of 9 (11%±10% actuarial) of the first patient group survives, while 10 of 19 (43%±13% actuarial) of the subsequently treated patients survive. Overall, at a median follow-up of > 400 days (minimum follow-up > 60 days), 11 of 28 (29%±10% actuarial) survive without evidence of neoplastic disease. T-cell depletion appears to decrease the incidence and modify the severity of GVHD from that historically encountered in such mismatched transplants, and allows successful partially-matched marrow transplantation for young leukemic patients. Extended family typing and nonrelated searches to identify the most suitable donor should be considered early in the disease course for leukemic patients who are candidates for marrow transplantation but who lack matched sibling donors.

P89 IN VITRO PURGING WITH ANTI-MYELOID MONOCLONAL ANTIBODIES IN ACUTE MYELOGENOUS LEUKEMIA: RESULTS OF AUTOLOGOUS MARROW TRANSPLANTATION. Edward D. Ball, Letha E. Mills, Christopher T. Coughlin, J. Robert Beck, and Gibbons G. Cornwell III, Departments of Medicine, Microbiology, and Pathology, Dartmouth-Hitchcock Medical Center, Hanover, N.H. 03756 Bone marrow was harvested from 7 patients with acute myelogenous leukemia (AML) in remission (3 patients in first, 3 in second, and 1 in third). Mononuclear cells were separated and treated with saturating concentrations of 2 complement-fixing monoclonal antibodies (MoAbs), PM-81 and AML-2-23 (J.I. 130:2937,1983 and PNAS 79:5374,1982), reactive with myeloid cell differentiation antigens, incubated with rabbit complement, and then cryopreserved. These MoAbs were chosen because they have broad reactivity with AML cells and react with committed myeloid progenitor cells but not with pluripotent progenitor cells. Patients were treated later with cyclophosphamide (60 mg/kg per day X2) and total body irradiation (200 cGy BID X3d) and then given infusions of MoAb-treated bone marrow (mean count: 3.0×10^7 cells/kg body weight). At the time of transplant, 3 patients were in second remission, 2 in third remission, and 2 in early first relapse. Full bone marrow reconstitution was observed in 6 patients, while one patient's platelet count never reached a sustained level greater than 20,000/ul despite the presence of megakaryocytes. Five of the 7 patients are surviving and well at 15, 9, 7, 4 and 1 month post-transplant, the longest survivors being in 2nd and 3rd remission at transplant. These results demonstrate that treating remission bone marrow with MoAbs to antigens that appear at the level of the CFU-GM does not interfere with pluripotential stem cell engraftment in patients with AML late in their disease and may contribute to improved survival.

P90 AUTOLOGOUS HEMATOPOIETIC RECONSTITUTION OF LETHALLY IRRADIATED DOGS USING IA-POSITIVE BONE MARROW CELLS, Ronald J. Berenson, William I. Bensinger, Dale Kalamasz, Friedrich Schuening, H. Joachim Deeg, and Rainer Storb, Fred Hutchinson Cancer Research Center, Seattle, Washington 98104

Previous work has demonstrated that bone marrow engraftment does not occur when lethally irradiated dogs receive autologous marrow depleted of Ia-positive cells by treatment with monoclonal antibody 7.2, which reacts with the canine Ia-antigen, and complement (Blood 65:819, 1985). We have now shown that successful hematopoietic reconstitution can be accomplished in lethally irradiated canine recipients with a relatively pure population of autologous Ia-positive marrow cells obtained with a newly-developed avidin-biotin immunoadsorption procedure. Buffy coat preparations of dog bone marrow containing 2-3 x 10^9 cells that were 10% Ia-positive cells were treated successively with 20 ug/ml antibody 7.2, 1:100 dilution of biotinylated goat anti-mouse immunoglobulin and passed over a 25 ml column bed of polyacrylamide beads containing 1 mg/ml avidin at a flow rate of 20 ml/min. A total of 180-220 x 10^6 adherent cells were recovered by mechanical agitation of which 75-87% were Ia-positive by immunofluorescence staining and FACS analysis. Infusion of this Ia-positive cell population into autologous recipients conditioned with 950 rad of whole body irradiation has produced successful marrow engraftment in three dogs. We conclude that Ia-antigen is expressed on canine hematopoietic stem cells. The recent development of monoclonal antibodies recognizing potential human pluripotent hematopoietic cells may make this avidin-biotin procedure useful in obtaining marrow stem cells for human BMT.

P91 APLASTIC ANEMIA SECONDARY TO A DEFECTIVE MARROW MICROENVIRONMENT: EVIDENCE FOR CORRECTION BY INTRAMEDULLARY TRANSPLANTATION OF CULTURED ALLOGENEIC MARROW STROMAL CELLS (MSC). H. Castro-Malaspina, J. Laver, B. H. Kushner, R. J. O'Reilly. Memorial Sloan-Kettering Cancer Center, New York, N. Y. 10021.

In vitro studies on marrow from a 42-year-old woman with severe AA revealed a quantitative defect of her MSC. Cocultures of her T-cells with normal allogeneic marrow cells showed no immune-mediated myelosuppressive activity. Two courses of antithymocyte globulin were unsuccessful. After preparation with TBI (1440 rad) and cyclophosphamide (120 mg/kg), she received a haplotype mismatched marrow transplant from her son. To prevent lethal GvHD, T cells were depleted by the soybean agglutinin (SBA) and E-rosetting technique, which also removes MSC. No evidence of engraftment could be documented after this transplant or following a second transplant from an uncle after further immunosuppression. This case differed from our other cases of graft rejection, in that host cells with donor specific or nonspecific suppressor or cytotoxic activity could not be identified. To correct a suspected stromal defect, MSC from the primary donor were established in vitro and then injected with the donor's T-cell-depleted hemopoietic cells directly into the host's marrow cavity. This approach was selected because MSC may not be transplantable by the intravenous route and, furthermore, are lacking in the SBA separated graft. No additional immunosuppression was given. Although death from herpes infection ensued 10 days post-transplant, morphologic identification of hemopoiesis and HLA identification of donor origin for MSCs grown from the sites of injection suggest that a defective human MSC population can be replaced by intramedullary transplantation of cultured normal stroma.

P92 HIGH DOSE RATE TBI AND CYCLOPHOSPHAMIDE AS CONDITIONING REGIMEN FOR BONE MARROW TRANSPLANTATION IN CHILDREN: EXPERIENCE OF PADUA BMT GROUP. Paolo Colleselli, Guido Sotti, Maurizio Belloni, Donatella Baronciani°, Chiara Messina, Roberta Destro, Marina Cavazzana, Maria Vittoria Gazzola, Luigi Zanesco. Cooperative Group for marrow transplantation. Padua, Italy. °Center for bone marrow transplantation. Pesaro, Italy.

Cyclophosphamide and TBI at low dose rate to a total of 1000 cGy are the commonly used conditioning regimen. However the problems of the long time needed by TBI and the Radiation Syndrome are amplified in children. We therefore used a conditioning regimen based on cyclophosphamide at 60 mg/Kg/day for 2 days and TBI at high dose rate to a total of 750 cGy according to Kim (Radiology 122: 523-525, 1977). 5 children, affected by AML (2) in first CR or ALL (3) in second or third CR, where treated with this intensive therapy followed by BMT from either a HLA-matched sibling (3), autologous marrow (1) or fetal liver (1). Ages ranged from 1 to 12 years. All the patients had complete marrow recovery, with the exception of the child transplanted with fetal liver. 2 patients (1 ALL, 1 AML) are alive and well at 24 and 30 months from BMT, 2 (1 ALL, 1 AML) are alive with disease at 6 and 8 months from BMT, 1 died for infection 3 months after fetal liver transplantation. In all cases TBI was delivered in about 30' without any important problem. No acute or delaied toxicity of TBI, included interstitial pneumonia, was observed. Since this conditioning schedule was,in our experience, well tollerated, we think it may be now indicated for BMT in children affected by AML in CR.

Supported by Grant n.850241544 from the Italian National Research Council.

P93 *PRODUCTION OF INTERFERON BY RECIPIENTS OF HLA-IDENTICAL SIBLING BONE MARROW TRANSPLANTS.* *M.A. Cooley, K.Brennan and K.Atkinson, Department of Haematology, St. Vincent's Hospital, Sydney, Australia.*

As part of a study of lymphokine biology in recipients of HLA-identical sibling bone marrow transplants, we investigated production of interferon gamma (IFNγ) by peripheral blood mononuclear cells (PBMC), and its presence in serum. Samples were obtained at times from 3 weeks to 4.8 years post transplant. Serum was stored at -85°C until assay. PBMC were separated on Ficoll-Hypaque density gradients, and cultured at 37°C for four days in the presence or absence of 20 µg/ml phytohaemagglutinin(PHA). Supernatants were then stored at -85° until assay. IFNs were assayed either by a bioassay measuring inhibition of virus growth in HEP-2 cell line, or by an IFNγ radioimmunoassay (RIA). None of 10 normal serum samples contained detectable IFN, and while 3/16 recipient serum samples contained IFN, this correlated with concurrent infections. Neither normal control (0/16) nor recipient (0/35) PBMC produced detectable IFN in the absence of PHA stimulation. Normal control PBMC produced 1.8±0.45 log U/ml IFN after PHA stimulation; 23/35 patient samples were within the normal range (mean±2S.D, 0.9-2.7 log U/ml), while 12 had low or undetectable IFN. IFN production did not correlate with time after transplant, presence of graft-versus-host disease, or infection. In 6 patient samples tested by RIA, the majority of the IFN produced was IFNγ. Production of this T lymphokine thus appears minimally impaired in most recipients of bone marrow allografts, in contrast to the profound depression of interleukin 2 production previously documented by ourselves and others.

P94 T-CELL DEPLETED MARROW TRANSPLANTS FOR CML. I.Cunningham, H.Castro-Malaspina, S.Gulati, N.Flomenberg, N.Collins, B.Shank, R.J.O'Reilly. Memorial Sloan-Kettering Cancer Center, New York, N.Y.

In an attempt to decrease the high incidence of GVHD seen in BMT for CML and the 30-40% early mortality, 20 adult patients received transplants of allogeneic marrow after removal of T-cells by soybean lectin agglutination and E-rosette depletion. Median age was 32 years (range 19-47). All but one had the Ph' chromosome; three had additional aneuploidy. All were in the chronic phase, at 9-120 (median 18) months after diagnosis. Fourteen had had splenectomy. Preparative cytoreduction consisted of fractionated total body irradiation (1320-1440 rads) followed by cyclophosphamide (60 mg/kg x 2). There was one graft failure. The 19 others engrafted, with 500 neutrophils attained at a median of 18 days (range 10-34). Splenectomized patients engrafted faster (median day 15 vs. 25) and required fewer transfusions. One patient developed Grade II GVHD and died of CMV pneumonia at 2 months. No other cases of acute or chronic GVHD or CMV were seen. Eighteen patients are well with performance status 100% at 2-20+ months. Recurrence of the Ph' chromosome was seen in a minority of cells in one non-splenectomized patient at 6 months, but there is no evidence of clinical relapse. We conclude that the use of marrow depleted of T-cells using soybean lectin agglutination and E-rosette depletion has usefulness as treatment for CML, and that the substantial decrease in the incidence of GVHD has decreasd the early morbidity and mortality which is characteristic of conventional BMT in CML. Patients with previous splenectomy appear to engraft faster and to have a shorter period at risk from aplasia. Further followup is needed to evaluate long-term survival.

P95 ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT) FOR THALASSEMIA MAJOR. Paolo Di Bartolomeo, Gabriele Di Girolamo, Francesco Angrilli, Alfredo Dragani, Marina Ciancarelli, Antonio Angelini, Antonio Iacone and Glauco Torlontano, Divisione di Ematologia e Cattedra di Ematologia, Pescara-Chieti, Italy.

Nine patients (pts) with homozygous ß-thalassemia aged 1 to 7 yr underwent BMT using their MHC-identical siblings as marrow donors. The median number of transfusions given before BMT was 30 (12-60). The preparative regimen consisted of busulphan (12 or 13 mg/Kg) and cyclophosphamide (200 mg/Kg). Pts were nursed in positive-pressure isolation rooms and received acyclovir orally from day -1 to day +120 as prophylaxis against herpetic virus infections. Cyclosporin was given to prevent GvHD from day -1 to day +365. The median marrow cell dose was 7.2 x 10^8/Kg (6-8.5). All pts achieved engraftment (median day +11). One patient died on day +12 of VOD and pneumonia. He had had hepatitis B before BMT. The remaining 8 pts achieved complete hematological chimerism as proved by cytogenetic examinations, red cell markers and analysis of Hb chain syntesis. In 1 patient progressive autologous reconstitution occurred 6 mo after BMT and transfusion regimen is required. The remaining 7 pts are living, well and hematologically normal 2 to 30 mo after BMT (median follow-up 15 mo). They have Hb levels greater than 10 g/dl, fetal Hb values less than 3% and Karnofsky scores of 100%. Of 8 evaluable pts 4 had grade 1 acute GvHD. None showed evidence of chronic GvHD or infection due to CMV or HSV.

Supported by CNR - Gruppo biologico Grant n. 84.00868.04 and MPI 40% - 1985.

P96 Limited Polymorphism of 3 Human Minor H Loci. W.L. Elkins, G.R. Pierson, K.S. Zier, University of Pennsylvania. It might be possible to avoid graft vs. host disease after transplantation between HLA matched sibs, were it possible to type and match the recipient and prospective donor for minor H antigens. Although this possibility cannot presently be tested, the problem can be approached by studying the polymorphism of minor H loci. A minor H locus with 2 or more alleles which are commonly expressed in the population could be of special importance as a source of the histoincompatibility between HLA matched sibs. Single H loci with just one very common allele would not be important, because they would so rarely provide the requisite imcompatibility between sibs. Using cold target inhibition of cytotoxicity against target cells, HLA matched with the effectors (Human Immunol. 7:117), we went on to determine the phenotypes of both members of 85 pairs of HLA matched siblings with respect to the minor alloantigen W1. 90% of the individuals were W1+, and only 10% of the pairs displayed incompatibility. We also generated other cytotoxic T cell probes, using recipients of HLA matched marrow grafts as the primary source of effectors to define other minor H antigens. Minor antigens were detected on host type cells that were lacking in the marrow donor, and these antigens were detected on cells from some other family members. However, the target antigens defined by these effectors were not expressed on cells from unrelateds, who shared restricting HLA antigens with the effector and target cells. Thus the minor H antigens that distinguish individuals in three sibships, do not commonly distinguish those in other sibships.

P97 DEFICIENT T CELL HEMATOPOIETIC GROWTH FACTOR PRODUCTION FOLLOWING BONE MARROW TRANSPLANTATION. Stephen G. Emerson, Colin A. Sieff, Steven C. Clark, Joel M. Rappeport and David G. Nathan. Harvard Medical School and Genetics Institute, Boston, MA.

T cell depletion of donor marrow prior to bone marrow transplantation (BMT) may result in decreased graft-versus-host disease, but may also cause more graft failure and graft rejection. This complication has been attributed to either 1) removal of an essential T cell hematopoietic growth factor, or 2) removal of a critical donor T cell required to kill radioresistant recipient T cells which destroy donor marrow cells. To analyze the role of T cell hematopoietic function post-BMT, we used sequential fractionation by panning to distinguish functional burst promoting activity (BPA) and granulocyte-macrophage colony stimulating activity (GM-CSA) from adherent cells, FcR+ cells and T cells from bone marrow donors and their recipients 3 weeks to 10 months post-BMT. Recipients' FcR+ and T cells produced severely reduced BPA and GMA-CSA as compared to their own transplant donor FcR+ and T cells. In addition, as determined by Northern blot hybridization, recipient T cells transcribed severely reduced levels of mRNA for human GM-CSF, a T cell hematopoietic growth factor known to possess both BPA and GM-CSA. This functional and molecular defect implies that the only T and FcR+ cells in BMT recipients which function as hematopoietic accessory cells are those donor cells that are infused in the original graft, and that the function of these cells is shortlived. Removal of these donor T cells prevents the production of hematopoietic growth factors such as GM-CSF which may lead to inadequte proliferation and and differentiation of infused stem cells, and thus to graft failure in T cell depleted BMT.

P98 PREVENTION OF GRAFT REJECTION IN T-DEPLETED ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT): PRECLINICAL STUDIES. A.H. Filipovich, G. Malilay, B. LeVasseur, R.M. Condie, E. Sevenich, University of Minnesota, Minneapolis, MN 55455.

Failure of sustained engraftment after T depleted BMT often leads to fatal complications. Resistance to engraftment, or rejection, may be mediated by host T or natural killer (NK) cells. In order to tailor a more immunosuppressive protocol for T-depleted BMT we performed in vitro studies to: 1) compare the effect of radiation protocols on NK activity and 2) investigate the ability of Cyclosporin A (CsA) or antithymocyte globulin(s) (ATG) to inactivate NK cells and presensitized T cytolytic cells (CTL). Peripheral blood mononuclear cells (PBMC) were cultured in conditioned medium (augmented NK) for 0 to 8 days and tested against K562 leukemic targets following variable irradiation protocols: single dose of 200,500 or 1000 cGy on day 0; fractionated dose: 165 cGy X 2 for 4 days; split dose: 1000 cGy day 0 followed by 500 cGy day 5 or 7. Although NK decreased with time and in all radiation schedules to 2.7-7.3% of original lytic units (LU), none of the radiation protocols completely ablated NK and the PBMC that remained viable 7-8 days after $\geq$ 1000 cGy were equal to, or more potent NK effectors than PBMC at the outset of culture. In separate studies, therapeutic concentrations of three lots of non-marrow toxic ATG decreased NK LU to 0-30% of untreated PBMC. The addition of autologous complement completely abrogated any residual NK LU. Preincubation with CsA over a wide range of concentrations as well as its addition directly to cultures had little effect on NK. As expected, ATG blocked CTL and CsA did not. We conclude that single-dose irradiation used 6 to 7 days prior to BMT and the infusion of ATG prior to, during and after BMT may promote engraftment of T-depleted marrow.

P99 CORRECTION OF IMMUNE DISORDERS BY HLA MATCHED OR MISMATCHED TRANSPLANTS, Alain FISCHER, Claude GRISCELLI. Hôpital des ENFANTS MALADES, PARIS.

Sixty five consecutive patients (aged from 2 months to 14 years) have received a bone marrow transplantation because of immune disorder between 1972 and Septembre 1985 in our institution. Overall results according to diagnosis are shown in the table. Patients from Groups II to V have received a conditioning including Busulfan (16 mg/kg) and cyclophosphamide (200 mg/kg) except for 3 of them who received a TBI. Mismatched bone marrow were depleted of T cells by E rosetting. No GVHd > grade II was observed in these patients. The actuarial survival curve for patients with SCID is 65 %, for patients (Groups II to V) who received an HLA identical bone marrow 48 % and a mismatched bone marrow 46 %. Death was related to pregraft abnormalities in half of the cases. No major difference in outcome is observed between HLA identical and HLA non identical BMT. However, mismatched BMT have been performed more recently than matched BMT. In addition failure of engraftment has been observed only in mismatched BMT performed in patients able to reject grafts (Groups II to V). The recent introduction for this group of the in vivo use of a monoclonal antibody anti LFA-1 (25/3) in order to prevent graft failure appears promising. Stable engraftment has been achieved in 3/3 patients (osteopetrosis 1, Wiskott Aldrich 2) without side effects while previously, engraftment was axhieved only in 1/8 comparable patients (excluding the inherited deficiency in LFA-1 molecule). A betterdefinition of the indications together with the improvement in infections -, GVHd -, and graft failures prevention cas allow the cure of immunodeficiency by HLA matched and mismatched BMT as well.

		NUMBER OF PATIENTS	ENGRAFTMENT	SURVIVAL (months)
		n=	n=	n= (months)
I Severe combined immunodeficiency	HLA identical	14	14	10 (24 → 150)
	HLA mismatched	12	11	8 (6 → 30)
II Combined immuno deficiency	HLA id.	7	6	2 (12 → 36)
	HLA mism.	5	2	2 (7 → 28)
III Wiskott Aldrich syndrome	HLA id.	6	6	3 (12 → 84)
	HLA mism.	3	3	2 (3 → 6)
IV Phagocytic cell diseases	HLA id.	8	8	4 (18 → 60)
	HLA mism.	5	2	2 (5 → 24)
V Ostepetrosis	HLA id.	3	3	2 (6 → 84)
	HLA mism.	2	1	1 (4 →)
		65	56	36

P100 BONE MARROW TRANSPLANTATION (BMT) FOR ACUTE LYMPHOBLASTIC LEUKEMIA (ALL): COMPARISON OF TWO HIGH DOSE CYTOSINE ARABINOSIDE (araC) FRACTIONATED TOTAL BODY IRRADIATION (fTBI) REGIMENS. J. Graham-Pole, G. Elfenbein, S. Gross, R. Weiner, M. O'Leary, D. Higby. Bone Marrow Transplant Units, University of Florida & Roswell Park Memorial Institute and the Pediatric Oncology Group (POG).

High dose cytoxan (Cy) & TBI are commonly combined to condition patients (pts) with refractory ALL for allogeneic BMT. This results in only about 35% longterm survival, because of both toxicity and relapse (rel). We recently achieved a 57% continuous complete remission (CCR) in such pts after substituting high-dose araC for Cy. In the current study we compared the toxicity and CCR rate of two sequential groups (gps) of pts with refractory ALL undergoing BMT following two araC containing regimens. The gps were comparable for age and stage and received the same supportive care. Gp I (n=11) received Cy 120mg/kg + araC 18gm/m + fTBI 26y x 6; Gp II (n=14) received araC 36gm/m + fTBI 26y x 6. Results are as follows:

	#	Early deaths	(%)		Survival	(%)	
Gp I	11	10	(91)	p 0.003	0	(0)	p 0.007
GpII	14	4	(29)		9	(64)	

Causes of death were: Gp I: sepsis 4, interstitial pneumonitis (IP) + graft-vs-host disease 5, encephalopathy 1; Gp II: sepsis 1, IP 2, hemorrhage 1. Additionally, 3 Gp I and 2 Gp II pts have had BM rel and I Gp II has had a testicular rel. Follow-up of the Gp II survivors post-BMT is 2,3,7,9,10,12,16,23, and 26 months. We conclude that for pts receiving BMT for refractory ALL, substituting araC in maximal dosage is less toxic and may be more effective than adding lower dose araC to a standard Cy/fTBI conditioning regimen.

Ref. (1) Herzig R, et al: Sem Oncol 12:184, 1985.

P101 CMV-NEGATIVE BLOOD PRODUCTS FOR PREVENTION OF CYTOMEGALOVIRAL PNEUMONIA (CMV-IP) IN SERONEGATIVE (CMV-Neg) TRANSPLANT RECIPIENTS--A STUDY IN 50 PATIENTS. Richard Harris, Steven Neudorf, Manley McGill and the BMT Team. Children's Hospital Medical Center and Hoxworth Blood Center, Cincinnati, Ohio 45229

CMV-Neg marrow recipients have a 20-30% risk of developing fatal CMV-IP in the posttransplant period. The use of blood products from CMV-Pos donors is probably the major source for the introduction of the virus into the patient. We have been exclusively using blood products obtained from CMV-Neg donors for transfusion support of CMV-Neg marrow recipients since 4/81. The patients and marrow donors were considered CMV-Neg if their CMV complement fixation titer was $\leq$ 1:4. Blood product donors were considered CMV-Neg if their CMV titer was $\leq$4 by ELISA. Prior to release each blood product was rescreened by indirect hemagglutination. As of October, 1985, 50 CMV-Neg marrow recipients have been entered onto the study; 12 of these had CMV-Pos marrow donors. 28 had leukemia (15 ANLL, 11 ALL, 2 CML), 5 lymphoma, 8 neuroblastoma, 3 other solid tumors, 3 aplastic anemia, 2 severe combined immunodeficiency, and 1 Hurler's syndrome. 36 had an allogeneic transplant, 3 syngeneic, 11 autologous. 13 had haploidentical donors; 19 received T-depleted marrows. 29 received total body irradiation. 12 had grade II-IV acute GVHD; 7 had chronic GVHD. 26 of the 50 patients are alive from 1 to 47 months (median 15 months) post BMT. None of the 50 patients have seroconverted or have developed evidence of CMV excretion or CMV-IP. Our attack rate of fatal CMV-IP in CMV-Pos recipients has been 28%; of CMV disease 43%. We conclude that the routine use of CMV-Neg blood products in CMV-Neg marrow recipients is not only practical but beneficial in the prevention of CMV-IP.

P102 ALL IN SECOND REMISSION-A CCSG STUDY OF MAINTENANCE CHEMOTHERAPY VS MARROW TRANSPLANTATION. R Harris, S Feig, P Coccia, P Warkentin, F Wiley, B Lampkin, J Kersey, H Sather, GD Hammond. The Children's Cancer Study Group, Pasadena, CA 91101

105 children with recurrent ALL were entered onto a CCSG protocol (CCG-181P) as of 9/85. Reinduction consisted of VCR/PRED/L-Asp (VPL) with a rescue reinduction of HD AraC/L-Asp for those patients failing VPL induction. Patients receiving maintenance chemotherapy were treated for 3 yr with intensive monthly cyclical pulses of cyclocytidine, Adriamycin, methotrexate, VCR, L-Asp and IT methotrexate on days 0-5, followed by a VCR/PRED pulse on days 21-25. Half of the patients also received low dose (50-75 cGy) TBI on day 0 of the 1st, 4th and 7th maintenance cycles. Those electing marrow transplant received preparation with Cytoxan 60 mg/kg x 2 and TBI 165 cGy BID x 8 doses. The VPL reinduction rate was 72%. Four of 9 receiving HD AraC/L-Asp rescue reinduction achieved CR. Of the 33 evaluable patients receiving maintenance chemotherapy, 6 (18%) (3 of 15 with TBI, 3 of 18 without TBI) are in CCR at 16+, 37+, 41+, 47+, 52+ and 64+ mo after entry onto study and 5 of these 6 are off therapy. Of the 27 patients transplanted in 2nd CR from matched donors with CTX and TBI, 12 (44%) are alive in CCR from 17 to 60 mo (median 33 mo) after transplant; 10 (37%) relapsed from 2 to 19 mo (median 8 mo) after transplant. Through amendment of this study, the CCSG is now studying BMT for ALL in 2nd CR utilizing as preparative therapy HDAraC 3 gm/m^2 BID x 12 doses and TBI 200 cGy BID x 6 doses (Coccia, Blood 5(Suppl 1):213a, 1984). Early results are confirming Coccia's excellent relapse-free survival and low relapse rate. Combining Coccia's patients and the newly entered CCG-181P patients, the RFS is 71% (12 of 17) and the relapse rate is 0% with a median f/u of 10 mo (range 1 to 54 mo).

P103 **The Significance of Gamma-Interferon (g-IF) in Patients with Severe Aplastic Anemia (SAA)**
W.Hinterberger, G.Adolf, U.Köller, W.Knapp, P.Bettelheim, K.Geißler, H.Gadner, B.Volc-Platzer, K.Lechner, Vienna, Austria

The possibility of auto-immunity in SAA has been supported by the observation of abnormally released g-IF by Dr+T cells, which is myelosuppressive. We studied a) lymphocyte subsets, b) the release of Colony stimulating Factor (CSF) and g-IF in PHA-activated mononuclear blood cells (MNC) and c) the effect of excess moAb directed against g-IF in clonogenic assays: In 11 untreated patients, the H/S ratio was obviously not correlated with g-IF released by MNC upon PHA. 10^6 MNC/ml of 6 untreated patients generated 240 IU/ml (med., range: 0-3200, normals: median 72, range: 0-240, n.s.), while MNC from 8 immunosuppressed patients continued to generate highly increased amounts of g-IF for up to 32 months. In the serum, g-IF was never detectable (before and after IS/normals). By neutralizing g-IF in MNC-PHA supernatants with excess moAb, CFU-GM growth of normal target bone marrow increased, indicating, that normal levels of CSF were masked by g-IF. Conversely, the numbers of CFU-MIX, BFU-E and CFU-GM failed to normalize or increase, respectively, in the presence of excess moAb. We conclude that, a) the H/S ratio obviously fails to correlate with the release of g-IF, b) upon PHA stimulation, CSF activity is masked by extremely high amounts of g-IF and c), growth of progenitor cells is not enhanced upon neutralization of endogeneous g-IF. Thus, the release of g-IF is under abnormal control in some patients, but the lack of enhanced colony growth upon neutralization of g-IF implies. that either other inhibitors are operating as well, or that g-IF does not act by continued suppression of stem cells.

P104 HAPLOTYPE IDENTICAL BONE MARROW TRANSPLANTATION FOR IMMUNODEFICIENCY USING MONOCLONAL ANTIBODY T CELL DEPLETION. R.Hong, R.Moen, S.Horowitz, M.Trigg, P.Sondel, J.Finlay, W.Ershler. U. Wisconsin, Madison 53792 and R. Billing, Westlake Village, CA.

Thirteen children with combined immunodeficiency diseases (11 ADA+ SCID and 2 ADA-SCID) were transplanted from a parent differing by 1 haplotype. A patient with Wiskott-Aldrich syndrome (WAS) received a transplant from his father who differed only at HLA-A. All marrows were depleted of mature T-cells by monoclonal antibody treatment with CT-2 and rabbit complement. Four children died within 14 days of the transplant from preexisting life threatening infection. One child did not engraft. Another engrafted only transiently (1 month). The remaining 8 patients are all well and all demonstrate evidence of T and B cell engraftment, with periods of followup ranging from 3 to 35 months. Graft versus host disease occurred in 4, of Grade I severity in 2, and Grade II in the others (Seattle criteria). In one patient, a thymus biopsy was performed 15 months after transplant and revealed normal morphology with ample numbers of thymocytes, good corticomedullary differentiation and Hassall's corpuscles. The patient with WAS was prepared for transplant by busulfan and cyclophosphamide. He demonstrates T cell chimerism, but following a transient engraftment of paternal platelets, his own have returned as demonstrated by antigen analysis, sizing and recurring thrombocytopenia. He has no infections, no eczema and his immunoglobulin pattern is normal, not revealing the high IgA and low IgM characteristic of WAS. Of the 10 surviving patients, ablative preparation was used in 5; all engrafted well. Of the remaining only 3 engrafted. The results show that monoclonal antibody depletion is an effective treatment for prevention of GVHD, haploidentical stem cells will traffic to and mature in the host thymus. Criteria for ablation need to be established.

P105 Abstract Withdrawn

P106 EMOTIONAL OUTCOME FOLLOWING BONE MARROW TRANSPLANTATION. Brett M.Jones & Ed Freed. Departments of Psychiatry & Haematology,St.Vincent's Hospital,Sydney.Australia.2010.

A prospective study is in progress to assess the emotional response to Bone Marrow Transplantation. Subjects are patients referred for B.M.T. who are English speaking and aged between 15 & 50. Emotional status is assessed using standardised psychological tests such as the Beck Depression Inventory and the Speilberger State Trait Anxiety Inventory & other ad hoc self rating scales designed to measure factors peculiar to B.M.T. To date 29 patients (23 M, 6 F) have been assessed prior to B.M.T. Their mean age was 31.3 yrs. Results indicated that anxiety was the main concern prior to transplantation, (68% scored abnormally high anxiety scores). 41% recorded low well being scores, indicating poor quality of life and 31% showed a mild to moderate depressive reaction. 19 subjects have been followed up at first discharge from hospital. Results generally showed a move in the direction of improved emotional status. However, all subjects showed a deteriorated body image post-transplantation. A small group of subjects have been followed up three months post-transplantation. There was no further change in emotional status at this time. The results suggested that there was significant anxiety about the outcome of the transplantation & that this was the most prominent emotional reaction. Despite the continued uncertainty in the first months post-transplantation the anxiety generally decreases. Though these early findings do suggest there is some psychological morbidity associated with B.M.T., they also show evidence of adaptability and resiliance when confronted with significant stress.

P107 FACILITATION BY LYMPHOKINES (LK) OF IMMUNO-HEMATOPOIETIC RECONSTITUTION OF IRRADIATED MICE, WITH AND WITHOUT BONE MARROW TRANSPLANTATION (BMT), E. Kedar, B. Tsuberi, M. Ayalon, Z. Lebendiker and B. Leshem, The Lautenberg Center, Hebrew University-Hadassah Medical School, Jerusalem 91010, Israel.

In this study we tested the possibility of accelerating the reconstitution of immuno-hematopoietic functions in immunosuppressed mice by the use of various LK. Three systems were studied: (a) sublethally-irradiated (450R) mice treated with LK (2-3xweek, for 2-3 weeks), without BMT; (b) lethally-irradiated mice transplanted with syngeneic BM ($2-10x10^6$ cells) in conjunction with multiple administration of LK; and (c) lethally-irradiated mice transplanted with BM cells that had been cultivated for 2-3 days with LK. As sources of LK we used partially-purified preparations obtained from culture supernatants of WEHI 3 leukemia cells (containing mainly IL-3), PMA stimulated EL4 leukemia cells and con A stimulated rat splenocytes (both containing a multitude of LK). The following parameters were assayed 2-8 weeks post irradiation: WBC count, colony formation (CFU-C, CFU-S), mitogenic and MLR responses, allocytotoxicity, NK activity, and antibody production. With the use of LK either in vivo or in vitro, a 50-600% increase, as compared to control groups not exposed to LK, was observed in nearly all the functions tested in the three systems at 3-5 weeks post irradiation. WEHI LK appeared to be the most effective and the splenocyte generated LK the least effective preparation. In preliminary experiments with highly purified LK, murine IL-3 enhanced whereas human IL-2 delayed reconstitution. It is suggested that the application in vivo and/or in vitro of certain LK might also be beneficial in clinical BMT.

P108 Abstract Withdrawn

P109 THE NUMBER OF RESIDUAL T LYMPHOCYTES MEASURED BY A LIMITING DILUTION ASSAY CORRELATES WITH THE DEVELOPMENT OF GVHD IN 32 T CELL DEPLETED BONE MARROW TRANSPLANT RECIPIENTS. N.A.Kernan, N.H.Collins, L.Juliano, T.Cartagena, B.Dupont, R.J.O'Reilly. Memorial Sloan-Kettering Cancer Center, NY, NY. A limiting dilution assay (LDA) for quantitation of T lymphocytes in human bone marrow (BM) was used to define a relationship between the number of residual T lymphocytes in a T cell depleted BM and the subsequent development of GvHD. 32 leukemic patients (mean age = 22 yrs.) who were durably engrafted and at risk for GvHD following transplantation of HLA identical BM depleted of donor T cells by differential agglutination with soybean lectin (SBA) and E-rosette sedimentation were evaluated. Each T cell depleted marrow was sampled prior to transplantation and evaluated for residual T lymphocytes by enumeration of E-rosettes and by LDA which determines the frequency of PHA-IL-2 responsive T lymphocytes in 1 x 10^6 bone marrow cells. In this series, 3 patients (25, 5 and 1.5 yrs.) developed grade II skin GvHD, 1 patient (12 yrs.) developed grade I skin GvHD and 28 patients did not develop GvHD. No patient developed gastrointestinal or hepatic GvHD. There was no correlation with the percentage of E-rosette positive cells in the bone marrow graft and the subsequent development of GvHD. However, a relationship did exist between the number of transplanted T ly nphocytes determined by the LDA and expressed as T cells per kilogram (kg) recipient weight, and the subsequent development of GvHD. Patients who did not develop GvHD (N=28) received a median of 3.98 x 10^4 (0.92-16) T lymphocytes/kg. Patients who developed GvHD (N=4) received a median of 23.99 x 10^4 (18.63 - 43.93) T cells/kg. No patient who received less than 1 x 10^5 T cells/kg developed GvHD (N=24). Of the 8 patients who received between 1 x 10^5 and 4.4 x 10^5 cells/kg, 4 patients developed skin GvHD. These data suggest that with this limiting dilution assay, the number of clonable T lymphocytes in a T cell depleted human BM correlates with the subsequent development of GvHD.

P110 REMOVAL OF B-LYMPHOMA CELLS FROM HUMAN BONE MARROW USING MONODISPERSE, MAGNETIC PARTICLES and ABRIN IMMUNOTOXINS., G. Kvalheim, Ø. Fodstad, A. Godal, K. Nustad, J. Ugelstad, A. Phil and S. Funderud. The Norwegian Radium Hospital, Montebello, 0310 Oslo 3 and NTH, Trondheim (J.U), Norway.

Conditions for removal of malignant B-cells from bone marrow using particles charged with monoclonal antibodies, as well as abrin immunotoxins were studied. B-cell specific monoclonal antibodies (IgM isotype) prepared in this laboratory were physically and chemically adsorbed to an improved generation of monodisperse, magnetic polymer particles. Burkitt lymphoma cells were mixed 1/9 with normal human bone marrow cells and charged particles (beads /tumor cells = 75/1) were added. The mixture was incubated in RPMI medium at 4°C and after 30 min the magnetic beads were removed with the aid of cobalt-samarium magnets. A tumour cell depletion in excess of 6 logs, as determined by a colony forming assay, was obtained with 3 cycles of treatment using beads charged with AB1(Pan B) and AB4(anti -HLA DR) antibodies. This treatment had no significant effect on the marrow progenitor cells, as measured in GM and GEMM assays. In the immunotoxin experiments, native abrin conjugated to the AB3 antibody (anti-HLA DR) was incubated with the cells for 2 hrs at 37°C in the presence of 0.1 M lactose. More than 3 logs of tumor cell kill was achieved at immunotoxin concentrations (100 ng/ml) that showed only slight toxicity to the bone marrow progenitor cells. Experiments in which the two different approaches are combined, are in progress.

P111 MARROW TRANSPLANTATION FOR THALASSEMIA, K. H. Lin, J. Y. Wang, Y.S. Lee, M.J. Lee, K.S. Lin, C. J. Lee, Taoyuan Provincial Hospital. Taiwan, R.O.C.

Ten patients with homozygous beta thalassemia aged from 1 year 7 months to 13 years underwent bone marrow transplantation from siblings or parents.The first case received 12 mg/Kg busulfan, 120 mg/Kg cyclophosphamide, and 300 rads total body irradiation before transplantation. He survived with graft 580 days after transplantation. The subsequent 9 patients received 16 mg/Kg busulfan and 200 mg/Kg cyclophosphamide. Two died of transplantation related complications on day 30 and 55. Seven survived 70-480 days after transplantation. Only 2 of 7 surviving patients had durable engraftment while 5 patients became thalassemic again and survived 70-130 days after transplantation. All 5 relapsed patients had more than 50 times prior blood transfusions. Post-transplant immunosuppression was with methotrexate or cyclosporin A. One patient developed grade 4 acute graft versus host disease and died of venocclusive disease 55 days after transplantation. One patient had chronic graft versus host disease which resolved without causing disability. The acturarial survival was 80% and the acuturarial disease free survival was 30%. These data demonstrate that bone marrow transplantation may cure thalassemic patients who had less blood transfusion.

P112 CMV SPECIFIC LYMPHOCYTE PROLIFERATION AND IN VITRO CMV IgG SYNTHESIS FOR DIAGNOSIS OF CMV INFECTIONS AFTER BONE MARROW TRANSPLANTATION.

Per Ljungman, Berit Lönnqvist, Gösta Gahrton, Olle Ringdén and Britta Wahren. Department of Medicine, Transplantation Surgery and Clinical Immunology, Huddinge Hospital, Huddinge, Sweden.

Twenty bone marrow transplant (BMT) recipients were monitored for lymphocyte proliferation and specific IgG production in vitro by cytomegalovirus (CMV) antigen in solid phase. Thirteen patients got a reactivated CMV infection as defined by virus isolation or serum IgG conversion. Lymphocyte proliferation and in vitro IgG production responses were significantly stronger in these 13 patients than in 7 CMV seropositive persons without ongoing CMV infection ($p=0.002$). CMV infection by these techniques was indicated at a mean of 45 days after BMT, which should be compared with a mean of 79 days before CMV growth in culture was detected ($p<0.05$). Lymphocyte proliferation and in vitro IgG production may thus be tools for diagnosis and monitoring of CMV infections in BMT recipients.

P113 TRANSFER OF ANTIGEN-SPECIFIC MEMORY CELLS FROM MARROW DONORS TO MARROW RECIPIENTS,

Lawrence G. Lum, Margaret C. Seigneuret, Nathan A. Munn, Neng-Ren Jin, and Rainer Storb, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

Few studies have shown transfer of antigen(Ag)-specific immunity from the marrow donor to the marrow recipient. Using in vivo and in vitro approaches we demonstrate that transfer of Ag-specific immunity plays a major role in immune reconstitution. Specific serum IgG antibody (Ab) titers to tetanus toxoid (TT), diphtheria toxoid (DT), and measles virus and in vitro production of IgG anti-tetanus toxoid (anti-TT) by peripheral blood lymphocytes (PBL) from long term marrow recipients were measured using ELISA assays. None of the marrow recipients were reimmunized to the test Ags postgrafting. Tetanus toxoid (a T-dependent B cell antigen) and Epstein-Barr virus (a T-independent polyclonal B cell activator) were used to induced anti-TT synthesis in 12 day cultures performed in RPMI 1640 supplemented with fetal calf serum [J Immunol 135:185,1985]. Our results show that IgG Ab titers to TT, DT, and measles virus were detectable in the serum of marrow recipients. 38 of 60 (63%) recipients had anti-TT titers in the normal range. 17 of 32 (53%) recipients had anti-DT titers in the normal range; and 18 of 33 (54%) recipients had anti-measles virus titers in the normal range. Six of 14 recipients had PBL that produced anti-TT after TT-stimulation and 9 of 21 recipients had B cells that produced anti-TT after EBV stimulation. We conclude that Ag-specific immunity can be transferred by marrow transplantation.

P114 B CELL GROWTH AND DIFFERENTIATION FACTOR RESPONSES OF B CELLS FROM MARROW TRANSPLANT RECIPIENTS.

Kosei Matsue, Lawrence G. Lum, and Rainer Storb, Fred Hutchinson Cancer Research Center, Washington, Seattle, WA 99104.

The ability of B cells to respond to B cell growth (BCGF) and differentiation (BCDF) factors was investigated in 20 marrow graft recipients. Five patients were studied at less than 100 days postgrafting (short-term) and 15 were studied at greater than 1 year postgrafting (long-term). Ten of the long-term patients had chronic graft-vs-host disease (GVHD). Normal T cells prestimulated with 12-O-tetradecanoylphorbol-13-acetate, washed and cultured for 3 days in phytohemmagglutinin to produce T cell supernatants (T-sup) containing BCGF and BCDF. Highly purified B cells were obtained from peripheral blood mononuclear cells depleted of T cells and monocytes. BCGF proliferative responses was measured by ^{3}H-thymidine incorporation after culturing B cells with Staphlococcus Aureus Cowan I antigen (SAC) for 3 days and then for an additional 3 days in the presence of T-sup. BCDF response was assessed by measuring IgG, IgM, and IgA by ELISA in supernatants from B cells cultured in the presence of SAC and T-sup for 10 days. All 5 short-term patients and one long-term patient with chronic GVHD showed decreased responsiveness of their B cells to any stimulation. Two long-term patients with chronic GVHD showed decreased responses of their B cells to both BCGF and BCDF although their B cells responded to SAC normally. Three out of 7 chronic GVHD patients and 2 out of 5 long-term healthy patients' B cells had normal responses to both SAC and BCGF but could not respond to BCDF. These data indicate that B cell defects which include the lack of responses to SAC, inability to respond to BCGF and/or BCDF, may represent B cells arrested at different maturational stages after marrow grafting.

P115 MISMATCHED BONE MARROW TRANSPLANTATION(BMT) USING SOYBEAN AGGLUTININ(SBA) PROCESSED T-CELL DEPLETED MARROW. K. Matthay, D. Wara, A. Ammann, A. Ablin, M. Cowan. Dept. of Pediatrics, University of California, School of Medicine, San Francisco, CA, 94143. A majority of children requiring BMT for leukemia, severe combined immunodeficiency (SCID) or other fatal conditions will not have histocompatible donors. The limitation to mismatched transplant for such patients has been fatal graft vs. host disease (GVHD) and lack of engraftment. We processed haplocompatible parental bone marrow with SBA and sheep erythrocytes to enrich for stem cells and reduce GVHD in 17 patients with immunodeficiency or leukemia. Myeloablation was used prior to transplant in 10/17 patients: 4/11 with SCID, 2 Wiscott-Aldrich syndrome, 1 short-limbed dwarf immunodeficient, and 3 with acute leukemia. All donors were parents and 15/17 were either Dr incompatible and/or reactive with the recipient in mixed lymphocyte culture. Eleven were complete haplotype mismatches. Mean bone marrow mononuclear cell dose was 1.7×10^8/Kg (range 0.2-5.0). Engraftment was achieved in 12/15 evaluable patients. Median time to engraftment was 13d in 8 evaluable patients with myeloablation (range 9-18d). Delayed lymphocyte engraftment occurred in 5 SCID patients who did not have myeloablation. Eight of 17 patients are now alive 4-41 mos post-BMT. In the surviving patients, T-cell reconstitution was demonstrable but subnormal. B-cell function was depressed for as long as 3 yrs post BMT, as shown by the lack of specific antibody production. No post-BMT GVHD prophylaxis was administered. Only 3/15 evaluable patients developed acute GVHD (Grade I); two had chronic GVHD (Grade I). Mismatched BMT, with the use of SBA processing and T cell-depletion, can result in successful engraftment without GVHD, thus providing new hope for patients without histocompatible donors.

P116 INCREASED INCIDENCE OF MAJOR HEMORRHAGE AND OF MORTALITY ASSOCIATED WITH LIVER BIOPSY PERFORMED IN ALLOGENEIC BONE MARROW TRANSPLANT (BMT) RECIPIENTS. Silberstein P, Watson K, von Beck G, Weisdorf S, Bloomer J, Haake R, Snover D, McGlave P, University of Minnesota Bone Marrow Transplant Group. Between 9/81 and 6/85, 102 recipients underwent diagnostic liver biopsy within one year of BMT. 8 of 102 (7.8%) developed major hemorrhage as a complication of liver biopsy and 3 of these patients (2.9%) died. This incidence of morbidity and mortality is significantly higher than that commonly associated with diagnositic liver biopsy in other clinical settings. We looked for predictors of bleeding by analyzing several patient characteristics. By logistic regression analysis, we found that BUN >35 mg/dl ($p<.004$) and platlet count $<90,000/mm^3$ ($p<.02$) independently predicted bleeding as a complication of liver biopsy in BMT recipients. All 8 patients who bled had leukemia and had received total body irradiation. 7 of 8 patients experiencing major hemorrhage were adults. Neither interval from transplant to biopsy, presence of abnormal coagulation parameters, nor type of biopsy performed correlated significantly with risk of bleeding. Extreme care should be exercised in the performance of diagnostic liver biopsies in allogeneic bone marrow transplant recipients.

P117 DIFFICULTY IN ESTABLISHING DIAGNOSIS FROM SINGLE-SITE LUNG BIOPSIES AND BRONCHIAL WASHING ANALYSIS IN CHILDREN WITH LEUKEMIA FOLLOWING MARROW TRANSPLANTATION. T. Miale, N. Mody, B. Dick, P. Nanavati, L. Mathew, R. Boedy, M. Steinberg, D. Davis, P. McConnachie, S. Chaudhary, G. Thatcher. Depts. Ped. & Pathol., South. Illinois Univ. Sch. Med., Springfield, IL, USA. Three children developed a severe respiratory distress at days +12, +11 and +11 respectively following allogeneic bone marrow transplantation from sibling donors. The first child was a 13 y/o Hispanic boy, transplanted in relapse of Philadelphia chromosome + ALL. At Day -14, a bronchial washing done for a streaky pulmonary infiltrate was negative for acid-fast bacilli. Miliary tuberculosis was discovered at post-mortem examination. A second child, transplanted in remission of null-cell ALL, developed severe hypoxia and hypercarbia on Day +11, but fully recovered following prolonged mechanical ventilation. An open lung biopsy showed a pattern of non-specific diffuse aveolar damage, compatible with respiratory distress syndrome. The third child was transplanted in remission of B-cell ALL and developed fatal Aspergillus flavus, CMV and H simplex type II pneumonia on Day +12. All had received cyclosporine, granulocyte transfusions and multiple antimicrobials. including amphotericin B. Hyperfractioned total body irradiation with lung shielding was used in latter two patients.

P118 Analysis of T Cell Repopulation By Limiting Dilution Methods After Bone Marrow Transplantation. Richard A. Miller and Marta K. Rozans, Boston University School of Medicine, Boston, MA 02118.

Restoration of protective T cell immunity after bone marrow transplantation can be difficult to evaluate, in that methods based on surface antigen "phenotyping" do not test functional competence, and conventional culture methods often reflect complicated interactions among cell populations that may regenerate asynchronously and to different extents. We have therefore used limiting dilution analyses (LDA) to estimate, in BMT recipients, the number of T cells able to respond to mitogen by clonal proliferation, by production of cytotoxic cells, or by secretion of Interleukin-2. Most patients in their first post-transplant year are found to have low levels of active T cells by LDA, even though many of them have regained normal levels of T cells as judged by FACS analysis. The disparity between the LDA data and the FACS results suggests that initial T cell repopulation may involve cells that are dysfunctional despite a normal antigenic phenotype. Patients in their second and subsequent years often recover T cells able to proliferate in clonal cultures, but only rarely recover normal frequencies of helper and cytotoxic cells. Many patients regain normal levels of PHA-induced mitogenesis in conventional cultures, despite their relative paucity of responding cell numbers; normal PHA values may in these cases reflect a balance between compensating deficiencies in the PHA-reactive cells and the cells that would ordinarily regulate proliferation.

P119 Impaired immunological long term recovery after marrow transplantation is associated with high donor and recipient age, infections, graft-vs-host disease and prednisolone treatment. T. Paulin and O. Ringdén, Huddinge Hospital, Stockholm, Sweden. Cellular immune recovery was studied *in vitro* in 67 patients who had survived for one month to more than 6 years after bone marrow transplantation (BMT). As test parameters phytohemagglutinin (PHA), concanavalin A (ConA), and anti-$\beta_2$microglobulin (anti-β_2m) were chosen because of relevant increases in lymphocyte response with time. Exponential functions (covariance analysis) were used and the results could be expressed both as total levels and as increase rate of response with time. Patients with younger donors had a markedly higher response level than patients with older donors, and also had a faster rate of increase with time. High recipient age was also significantly associated with depressed levels of immunological response. Patients with chronic GVHD had a more severely impaired response, whereas acute GVHD did not give significant differences because of the few cases studied. Both patients with chronic and acute GVHD showed a negative development over time in the anti-β_2m test. Patients with bacterial infections seemed to have more depressed responses than non-infected patients. Patients undergoing cytomegalovirus infections had lower response levels for all three mitogens, for PHA the increase rate was also lowered. Patients treated with prednisolone for grade I GVHD had depressed stimulations for up to 1½ year post BMT compared to patients without GVHD and not receiving prednisolone. Lymphocytes from patients receiving a low marrow cell dose also showed impaired response levels and rate of recovery compared to the high dose group.

P120 Close Linkage of the Wiskott Aldrich Syndrome with a Polymorphic Marker from the Proximal Short Arm of the X Chromosome

Monica Peacocke, and Katherine Siminovitch - the Metabolism Branch, N.C.I. and the Department of Medicine, Toronto Western Hospital

The Wiskott Aldrich Syndrome (WAS) is an x-linked recessive disorder characterized by thrombocytopenia, eczema and immunodeficiency. We have used twenty restriction fragment length polymorphic markers from the X chromosome to examine twelve kindreds with female WAS carriers and demonstrated close linkage (lod of 4.37 at θ of .05) with the polymorphic sequence, 58.1 (DXS14 locus, Xp11 - cent.). From this linkage data we estimate a genetic distance of 4 centimorgans between WAS and DXS14 and suggest that the WAS locus lies on the proximal short arm of the X chromosome. This finding is of potential significance for prenatal diagnosis and carrier state detection and should facilitate further studies directed at precise localization and isolation of the WAS gene.

P121 **A STRATEGY FOR THE DEVELOPMENT OF TARGETED HIGH DOSE COMBINATION CHEMOTHERAPY PROGRAMS FOR SOLID TUMORS AND LYMPHOMA, William P. Peters, Gregg A. Olsen, Jon P. Gockerman and Robert C. Bast, Jr. The Duke University Medical Center, Durham, NC 27710.**

The development of disease-oriented high dose chemotherapy combinations is predicated on selecting agents having independent activity in the targeted disease, which are non-cross resistant, and have non-overlapping, non-myelosuppressive toxicities. However, modification of high dose multiple drug regimens may result in unexpected toxicities. In Phase I trials we have evaluated two targeted multiple drug combinations which differ from previously reported regimens by modifying only one drug. The first trial (CPA/cDDP/melphalan) targeted to melanoma and breast cancer was undertaken to evaluate the unexpected melphalan-induced nephrotoxicity previously reported to complicate CPA/cDDP/BCNU/melphalan administration. This study shows that removal of carmustine is associated with reduced tubular dysfunction. However, previously unappreciated high grade proteinuria was noted. This data suggests that the severe nephrotoxicity of CPA/cDDP/BCNU/melphalan may have resulted from overlapping nephrotoxic effects in different portions of the same organ (tubular vs glomerular). Cardiotoxicity also appears to be prominant in this regimen. A second trial targeted toward lymphoma evaluates the addition of VP-16 to fixed doses of CPA/cDDP/BCNU. This addition results in expected increased mucositis which has not been dose-limiting. Phase I trials modifying a single drug in a defined multiple drug regimen provides a rational and evaluable strategy for the development of targeted preparative regimens.

P122 B CELL LYMPHOPROLIFERATIVE DISORDERS (LPD) FOLLOWING T-DEPLETED HAPLOIDENTICAL BONE MARROW TRANSPLANTION (BMT), D. Pietryga, R.S. Shapiro, B.R. Blazar, J. Greenberg, J.H. Kersey, N. Ramsay, K. McClain, A. H. Filipovich, Univ. of Minnesota, Minneapolis, 55455.

Four children who received T depleted BMT from family donors developed fatal LPD 2-49 months later. Three patients (pts.) had BMT for immunodeficiencies and one for leukemia. T depletion methods included soybean lectin agglutination (1), E rosetting (1), immunotoxins (2). Prior to tumor diagnosis 3 pts. had demonstrated predominant or complete donor engraftment and 1 pt. had autologous marrow recovery. All pts. were discharged from the hospital in good condition prior to developing LPD. Post-BMT all 4 pts. had reconstitution of natural killer activity and 3/4 demonstrated T cytotoxity against EBV-infected cells in vitro. All 4 pts. had serologic evidence for primary (2) or reactivated (2) EBV infection. Persistent fevers in all 4 pts. eventually led to diagnostic biopsy of primary tumors in the lung, appendix, brain or liver. DNA extracted from tumors of 3 pts. was probed for viral genomes, host and donor restriction fragment length polymorphisms (RFLP), and mu heavy chain immunoglobulin gene rearrangement. 20-50 copies of EBV genome/10mcg of DNA was detected in all tumor specimens. Two cases had host RFLPs and one-donor RFLPs in all tissues studied. Two cases studied showed mu rearrangement indicative of monoclonal proliferation in primary and metastatic tumors (1 host, 1 donor derived) Combinations of antiviral therapy, immunotherapy and conventional chemotherapy were unsuccessful, indicating that more effective means must be sought to prevent this lethal posttransplant complication.

P123 CHARACTERIZATION OF A MOUSE-MONOCLONAL ANTIBODY DIRECTED AGAINST MEDULLOBLASTOMA, Feickert, H.J., Pietsch, T., Appelhans, K. AND Riehm, H., Medizinische Hochschule Hannover, Zentrum Kinderheilkunde, 3000 Hannover 61, Federal Republik of Germany.

A [C57 BL6]xF1 (female) mouse was immunized with whole cells of an established cell line derived from a medulloblastoma (TE-671) and monoclonal antibodies (mAbs) produced following standard prodedures. One clone (T-199) showing restricted reactivity in initial screenings was selected and further characterized after five cycles of subcloning by limiting dilution techniques. mAb T-199 is of the IgG1 isotype. Specificity analysis utilizing a panel of tumor cell lines indicated that T-199 recognizes a cell surface determinant restricted to neuroepithelial tissues. Besides TE-671, 5 of 9 neuroblastoma cell lines, 2 of 9 melanomas and 1 retinoblastoma expressed the T-199-antigen. Analysis by immunofluorescence methods on frozen tissue sections revealed large amounts of the antigen on the cell surface of tumors (neuroblastomas and gliomas). The antigen could not be demonstrated in normal tissues with the exception of normal brain where minimal amounts were detected. Whether the antigen is located within normal brain cells or on the cell surface is currently under investigation as well as its biochemnical properties.

The mAb T-199 could be useful for the diagnosis of tumors derived from neuroepithelium (especially neuroblastomas), the differential diagnosis of small round cell tumors and purging of bone marrow for autologous bone marrow transplantation.

P124 IMMUNOLOGICAL RECONSTITUTION AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION, Hiroshi Sao, Yasuo Morishima, Tatuya Yamauchi, Toru Tahara, Shinichi Mizuno, Hironori Yamada, Yoshihisa Kodera and Satoshi Yoshikawa, Nagoya 1st Red Cross Hosp., 1st Dept. of Int. Med. Nagoya Univ., NAGOYA, Japan

For allogeneic bone marrow transplantation (allo-BMT), severe infections such as interstitial pneumonia remain as major fatal complications. These complications are clearly attributable to the immunodeficiency which may be partly the results of insuficient reconstitution of the recipient's immune systems. In ordor to examine the recovery of immunological abnormalities after allo-BMT, T cell subpopulations, lymphocyte gamma interferon and IL-2 production capacity and blastgenetic response to mitogen were compared between 19 adult patients within 1 year after BMT and 10 adult patients living more than 2 years after BMT. Abnormality of T cell subpopulation such as high ratio of OKT-8, Ia and Leu-7 positive cells, and low ratio of OKT-4, 4A and 9.3 positive cells which were observed early after BMT returned to be within normal range after 2 years in almost all cases except slightly higher ratio of Leu-7 positive T cells. Functional activities of lymphocytes such as gamma interferon production capacity, PHA, ConA and PWM srimulation and IL-2 production capacity which were remarkably decreased within 1 year after BMT have almost recovered to normal range after 2 years.

P125 EFFECT OF DT-POLIO IMMUNIZATION POST BONE MARROW TRANSPLANTATION, E. Fred Saunders, and Stanley E. Read. Divisions of Hematology and Infectious Diseases, Hospital for Sick Children and Department of Pediatrics, University of Toronto, Toronto, Canada.

Eight children (4 ALL, 2 ANLL, 2 AA) were immunized with DT-polio vaccine post BMT. All were well and free of GVHD. Immunization was done at 12, 14, 16 and 28 months post BMT using 1.0 ml. S.C. of fluid vaccine (Connaught Laboratories, D10Lf, T10Lf per ml. Salk type polio). Antibodies to diphtheria, tetanus and polio types 1, 2 and 3 were assayed before each immunization and annually. Levels of diphtheria and tetanus antibody >0.01 units and of polio antibody >1:8 were considered protective. Prior to immunization only 2 patients had protective antibody levels to any of the above antigens. Following immunization all patients developed protective antibody levels against antigens tested, with the exception of 1 who failed to respond to diphtheria and 1 patient with a borderline response to polio type 3. We conclude that patients do not have protective levels of antibody to diphtheria, tetanus and polio post BMT. Immunization starting 12 months post BMT is effective in producing immunity in patients free of GVHD.

P126 **THYROID DYSFUNCTION (TD) FOLLOWING BONE MARROW TRANSPLANT (BMT): LONG-TERM FOLLOW-UP OF 53 PEDIATRIC PATIENTS,** R.S. Shapiro, L.L. Robison, T.H. Kim, O.H. Pescovitz, R. Haake and N.K.C. Ramsay, University of Minnesota, Minneapolis, MN 55455

Long-term follow-up of thyroid function was assessed in 53 pediatric patients (pts) after successful BMT for aplastic anemia (AA) (24 pts), acute nonlymphocytic leukemia (21 pts), or acute lymphocytic leukemia (8 pts). Median age at BMT was 11.1 years (range 1.5-20.4) and pts were followed for a median of 46 months (range 16-80). Pre-BMT conditioning consisted of high dose chemotherapy in all pts followed by single dose total lymphoid irradiation (TLI) (750 cGy) for pts with AA (group I) or single dose total body irradiation (TBI) (750 cGy) for those with leukemia (group II). The two groups did not differ significantly in age, sex, graft versus host prophylaxis (GVHP), GVH disease (GVHD), or follow up. TD occurred in 13/24 pts in group I and 6/29 pts in group II. 11/13 group I pts with TD had compensated hypothyroidism (CH) (↑TSH only) and 2 primary hypothyroidism (↑TSH, ↓T4 index). All 6 group II pts with TD had CH. The median time to develop TD for Group I was 392 days (range 98-1557) and 725 days (range 524-1158) for group II. 4 year life table estimated incidence of TD for groups I and II was 55% and 24%, respectively ($p<0.01$). TD did not correlate with age at BMT, sex, or GVHD in either group. In group I, 9/12 pts (75%) who received GVHP including prednisone remained euthyroid compared to 2/11 pts (18%) who received methotrexate alone $p<.05$. This association was not seen in group II. Pts with AA who receive TLI as part of their pre-BMT conditioning are at significantly greater risk of developing TD than pts with leukemia receiving TBI. GVHP regimens which contain prednisone correlate with a significantly lower incidence of TD in AA pts but not in leukemia pts.

P127 CLINICAL CORRELATES OF UNUSUAL CIRCULATING LYMPHOCYTES APPEARING POST MARROW TRANSPLANTATION. BR Smith, JM Rappeport, KA Ault. Brigham & Women's Hosp, Boston, MA. Dual immunofluorescence FACS analysis of blood lymphs following BMT demonstrates the existence of 3 unusual phenotypically unique cells: 1) a T cell which is CD3+ CD8+ but CD5 negative; 2) a natural killer (NK) cell which is Leu11+ CD3- but also CD8 positive; 3) a B cell which is Leu12+ IgM+ IgD+ but also CD5 positive. In normals all 3 of these cells are very rare (<5% of blood lymphs) but post BMT often represent > 50% of circulating lymphs. The CD8+ NK cells have the same cytolytic capacity against K562 as CD8- NK cells. The CD5- CD3+ CD8+ T cells have significantly less proliferative capacity as well as less lectin mediated cytolytic capacity compared to CD5+ CD3+ CD8+ T cells isolated from the same individual. A comparison of pts who developed either >grade II acute GVHD and/or chronic GVHD with pts not developing GVHD demonstrated that during the 1st 100 days post BMT the mean number of CD8+ NK cells was 30/mm3 in those developing GVHD but 5 fold more frequent (149/mm3) in pts destined not to develop GVHD ($p<.05$). By contrast, mean number of CD8+ T cells was 610/mm3 in pts developing GVHD but 3 fold less frequent (233/mm3) in pts not developing GVHD ($p<.05$). While surface expression of HLA-DR, Leu7, and Leu15 did not clinically differentiate pts, significant expression of IL2 receptor in the first 60 days post BMT has so far been observed only in pts undergoing graft rejection or developing severe GVHD, and has only been expressed on cells of CD3+ CD8+ lineage. We conclude that cells of unique surface phenotype that are rare in normals may constitute greater than 50% of post BMT lymphocytes and that these cells have unique functional and clinical correlates.

P128 INTERSTITIAL PNEUMONITIS FOLLOWING TOTAL BODY IRRADIATION FOR BONE MARROW TRANSPLANTATION USING LOW DOSE-RATE. Guido Sotti, Alberto Rigon, Maria Luisa Friso, Giovanni Scarzello, Paolo Colleselli, Paola Polchi° and Fulvio Calzavara. Co-operative Group for Marrow Transplantation- Padua, Italy. °Center for Bone Marrow Transplantation - Pesaro, Italy.

From June 1983 until April 1985 at Padua Hospital Department of Radiation Therapy, 35 patients with leukemia (15 CML, 12 ALL, 8 AML) received 1.000 cGy total body irradiation (TBI) in a single fraction. The TBI technique and dosimetry were based on the procedures used at the Royal Marsden Hospital (Lawrence et al.). The dose-rate was very low, 2-3 cGy/min; the lung dose was 1.000 cGy $\pm$ 7% (range 930-1070 cGy). The day after TBI the patients have undergone bone marrow transplantation (BMT) by the Center for BMT of Pesaro. 4 patients (11.4%) developed interstitial pneumonitis and 3 died (8.6%). One patient had Cytomegalovirus IP, confirmed histologically and died 75 days after grafting. 3 cases of idiopathic pneumonitis were seen; 2 died 34 and 116 days after BMT. The incidence of IP reported here is lower than that described by other transplant groups. As demonstrated by Barret and others, we confirm that dose rate may significantly affect the incidence of IP.

Supported in part by Grant n.850241544 from the Italian National Research Council.

P129 IMMUNE RECONSTITUTION AFTER TRANSPLANTATION WITH BONE MARROW TREATED EX VIVO WITH IMMUNOTOXIN IT 101. G.M. Spurll and A.A. Fauser.
Division of Hematology, Royal Victoria Hospital, McGill University, Montreal, Canada.

The removal of T cells from allogeneic bone marrow to prevent graft versus host disease may delay the generation of functional lymphocytes from the donor marrow. To examine this question, we have studied lymphocytes from patients undergoing transplantation to determine the lymphocyte phenotype and ability of T lymphocytes to support pokeweed mitogen (PWM) stimulated immunoglobulin synthesis. Three patients have been studied to day 90 post transplantation, two of whom received marrow depleted of T lymphocytes with IT 101, a pan-T monoclonal antibody coupled to Ricin-A-chain. All patients show decreased numbers of surface immunoglobulin and T3-bearing lymphocytes. Only the patient who received non-IT 101-treated marrow shows normal numbers of T4 cells at day 90. All patients show increased numbers of OKT8 and Leu 11 positive cells. The patient who received marrow without IT 101 depletion shows poor PWM response at 60 days, but a normal curve at 90 days. One of the patients with IT-101 depletion shows increased T cell activity at 60 and 90 days. The other, who continues to have very low (6%) T4 cells at 90 days, shows no response to PWM. These preliminary data may indicate that the course of immunological reconstitution is altered in patients receiving T-lymphocyte depleted bone marrow.

P130 DOSE RATE AND FRACTIONATION:RELATIVE IMPORTANCE IN RADIATION FOR BONE MARROW TRANSPLANTATION.
Nancy Tarbell, David Amato, Peter Mauch and Samuel Hellman
Joint Center for Radiation Therapy, Harvard Medical School, Boston, Mass. 02115

The optimal dose rate and fractionation schedules for total body irradiation TBI are presently unknown. This study compares several fractionation and dose rate schedules that are currently in use clinically in an attempt to define the optimal therapuetic ratio. C3H/HeJ were given TBI and the bone marrow survival fraction was calculated using CFU's assay. Irradiation was given as low dose rate (LDR) or high dose rate (HDR) at in single fraction (SF) and fractionated (FX) regimens. The results indicate effect of fractionaton for the normal bone marrow stem cells. To study the effects of oral-esophageal and pulmonary toxicity, mice were given upper half body irradiation and the LD 50's determined. The fractionation regimens were given as either 120 CGy given three times daily 200 CGy given twice daily or 200 CGy daily. For the oral-esophagus, the single SF LD 50/30 was 1488 CGy (1467-1508). LD 50/30 for the SF LDR group was 2487 (2434-2545), an increase of 1000 rad (p 0.001) demonstrating a strong dose rate effect. All fractionated regimens had higher LD 50's (2934-3262) than those achieved by LDR alone. There was no dose rate effect once fractonation was used.
Pulmonary lethality was evaluated using the LD 50/180. SF again, a large dose rate effect was demonstrated. The LD 50/180 for the 200 CGy HDR group was 2410 compared with 2842 (p=.001) for the 200 LDR group.

Our results indicate that fractionation appears to improve thoracic tolerance over low dose rate in every case. For oral-esophageal tolerance, dose rate has no effect once regimens allowing full repair with small fraction sizes of 120 or 200 CGy are used. However, there appears to be a dose rate effect for the lung when using 200 CGy this effect was no longer seen with 120 CGy indicating that 200 rad fractions are off the shoulder for pulmonary tolerance and do not allow full repair, but 120 CGy do. these results and their implications for optimizing the radiation schedule in human TBI for BMT will be discussed.

P131 IMMUNOMAGNETIC PURGING OF T CELLS FROM HUMAN BONE MARROW, F.Vartdal V.Bosnes, G.Gaudernack, G.Kvalheim, T.Lea, J.Ugelstad, D.Albrechtsen
The National Hospital and The Norwegian Radium Hospital, Oslo and SINTEF, Trondheim, Norway

Bone marrow T cells were rosetted with magnetic monosized polystyrene microspheres coated with T cell specific monoclonal antibodies. Rosetted T cells were subsequently removed from the non-T cell fraction of bone marrow by help of a magnet. This immunomagnetic separation procedure is carried out in less than 40 minutes and effectively and reproducibly removes T cells from bone marrow mononuclear cell suspensions, leaving maximum 0.025 percent sheep-red-blood-cell rosette forming (T) cells and less than 0.02 percent T cells as detected by a T cell limiting dilution assay. The efficiency of the T cell depletion procedure is further supported by flow cytometry data and by abrogation of interleukin 2 producing capacity in the T cell depleted bone marrow cells. This purging procedure is accomplishing approximately 60 percent recovery of non-T cells in the bone marrow mononuclear cell fraction and does not disturb the growth potential of bone marrow progenitor cells as assayed by hematopoetic stem cell assays.

P132 ENGRAFTMENT OF BONE MARROW STEM CELLS IS FACILITATED BY MONOCLONAL ANTIBODY PRETREATMENT OF UNIRRADIATED HOSTS. Michael Voralia*, Michel Sadelain*, and Thomas G. Wegmann, MRC Group on Immunoregulation and Department of Immunology, University of Alberta, EDMONTON, Alberta, Canada T6G 2H7

Bone marrow transplantation is rapidly gaining acceptance as a viable therapeutic modality in the treatment of various lymphohemopoietic diseases. It will also be the route of choice for the genetic therapy, via the reintroduction of genetically altered stem cells, of certain inherited disorders. However, a benign method for the engraftment of stem cells that does not rely upon lethal total body irradiation or toxic drugs, which lead to various complications, is lacking. To this end, we have developed a murine model that utilizes pretreatment of unirradiated F_1 hosts with monoclonal anti-major histocompatibility complex (mAb) prior to transplantation of either syngeneic or parental semiallogeneic bone marrow cells. Engraftment of donor stem cells is readily quantitated by Glucose phosphate isomerase (GPI) isozyme analysis of peripheral blood lysates. The results indicate that approximately 400ug of anti-Class I mAb administered to adult $(C3HxB6)F_1$ mice a week prior to transplantation of 3.10^7 B6 or (C3HxB6)-Gpi congenic F_1 bone marrow cells lead to a permanent donor engraftment of 100% and 40%, respectively, without accompanying immunodeficiency. These data thus suggest that host pretreatment with anti-host mAb negates the need for total body irradiation or toxic drugs for the engraftment of histocompatible or histoincompatible donor bone marrow cells. We are currently investigating the parameters required to obtain complete syngeneic engraftment as well as the applicability of this protocol in achieving totally allogeneic engraftment. *Supported by the Alberta Heritage Foundation for Medical Research.

P133 POSITIVE EFFECT OF TOTAL PARENTERAL NUTRITION ON OUTCOME OF BONE MARROW TRANSPLANTATION IN PREVIOUSLY WELL NOURISHED PATIENTS. Weisdorf SA, Lysne J, Wind D, Haake R, Schissel K, Sharp H, Goldman A, McGlave P, Ramsay N, Kersey J,. Departments of Pediatrics and Medicine, University of Minnesota, Minneapolis, MN.

Seven parameters of clinical outcome were evaluated to assess the impact of providing TPN during cytoreductive therapy and for 4 weeks after BMT to normally nourished patients. 137 patients (127 with malignancy, 32 autologous, 105 allogeneic) meeting criteria for normal nutritional status were stratified for age and for autologous versus allogeneic transplant and randomized to receive TPN during cytoreduction or to receive 5% dextrose with electrolytes, minerals, trace elements and vitamins. Average total daily calorie intake was 1.36 x basal energy expenditure (BEE) and average total protein intake was 1.75 g/kg/day in the TPN group and 0.72 x BEE with 0.57 g/kg/day protein in the control group. 40/66 control patients required TPN on the basis of nutritional depletion prior to discharge. Minimum follow-up was 3 months and median was 1 year. By life table analysis, survival (p=0.28), time to relapse (p=.022), and disease-free survival (p=.033) were significantly longer in the TPN group. Engraftment, duration of hospitalization, and incidence of graft-vs-host disease and bacteremia were not different. We observed that providing a nutritionally replete milieu during BMT had a positive effect on long-term outcome. We conclude that prophylactic nutritional therapy is indicated even for well-nourished individuals during cytoreduction and BMT.

P134 A PHASE I-II STUDY OF BIALKYLATOR CHEMOTHERAPY (BACT) WITH HIGH-DOSE THIOTEPA (TT) AND CYTOXAN AND AUTOLOGOUS BONE MARROW REINFUSION IN PATIENTS WITH REFRACTORY LYMPHOMA AND DISSEMINATED CARCINOMA. S. Williams, J. Bitran, M. Ratain, M. Egorin, J.Sinkule, R. Jacobs, J. Beschorner, C. Schroeder, S. Purl, K. Royston. University of Chicago and Michael Reese Medical Centers, Chicago, IL and University of Maryland Cancer Center, Baltimore, MD.

This study was undertaken to determine the toxicity and efficacy of BACT in patients (pts) with disseminated cancer, (untreated and previously treated) and to determine the pharmacokinetics of high dose TT. Bone marrow was harvested from the pts in the amount of 2-5x10^8 nucleated cells/kg and stored frozen in DMSO. On days -5,-3,-1, cyclophosphamide 2.5gm/m^2 was given with escalating doses of TT with 3 pts at 1.8mg/kgs, 2 pts at 3.6mg/kg . Bone marrow was reinfused on day 0. Three pts had lung carcinomas, 1 each diffuse histiocytic lymphoma, Stage IV melanoma. Two pts expired during their course of therapy; one with CMV pneumonitis, one with disseminated aspergillus. The mean time to hematologic recovery was 16 days. Toxicities included nausea/vomiting, diarrhea, stomatitis, skin rash andinfections. All pts to date have shown objective response (2CR,3PR). TT concentrations were determined by gas chromatography in 2 pts and fitted to a 2-compartment model. The peak was 2.2,5.1ug/ml; with α half-life of 5.3,15' and β half-life of 2.4,2.6°. The clearance was 3.9,7.1mg/min/kg. There are substantial toxicities associated with BACT, yet BACT appears to be effective in the treatment of patients with disseminated cancer.

P135 **TREATMENT OF ADVANCED HODGKIN'S DISEASE (HD) WITH HIGH-DOSE CYCLOPHOSPHAMIDE (CY) TOTAL BODY IRRADIATION (TBI) WITH OR WITHOUT INVOLVED-FIELD RADIATION (IFR) AND AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT).** S.N. Wolff, G.L. Phillips, J.W. Fay, H.M. Lazarus, R.H. Herzig and G.P. Herzig, Vanderbilt University, Nashville, TN 37232, University of British Columbia, Vancouver, B.C. V5Z IM9, Baylor University Medical Center, Dallas, TX 75246, Case Western Reserve University, Cleveland, OH 44106, Cleveland Clinic Foundation, Cleveland, OH 44106 and Washington University, St. Louis, MO 63110.

Twenty-three patients (pts) with HD have been treated with CY, TBI and cryopreserved ABMT. Eligible pts received IFR (2000 rads) over 14-24 days to tumor bulk prior to ABMT. All pts had advanced or progressive disease. Prior chemotherapy was substantial with 8 (range 5-11) being the median number of drugs per pt. The median age of all pts was 31 years (range 15-57). Overall, 16 pts (70%) achieved a CR, 3 pts (13%) a PR and 4 pts (17%) NR. Of the pts in CR, 3 died of toxicity and 2 have relapsed; all pts with PR or NR have died. For the entire group and for the 17 pts who received IFR, the probability of disease-free survival at 37 months post-ABMT is 42% and 54% respectively with all deaths counted as relapse. Prolonged myelosuppression, especially thrombocytopenia, was observed in some pts. Although toxicity was formidable, intense marrow ablative chemoradiotherapy has achieved a high proportion of disease-free survival in pts with advanced HD not expected to benefit from more standard approaches. This study is ongoing with future efforts aimed at decreasing the hematopoietic toxicity and defining the optimal preparative regimen.

Correction of Genetic Diseases

P136 **A PREPARATIVE REGIMEN OF COMBINATION ALKYLATING AGENTS WITH AUTOLOGOUS BONE MARROW TRANSPLANT (ABMT) FOR SOLID TUMORS: PHASE II** **K. Antman, JP Eder, S. Schryber, J. Andersen, WP Peters, WD Henner, R. Finberg, AD Elias, T. Shea, D. Wilmore, W. Kaplan, M. Lew, MS Kruskall, K. Anderson, B. Gorgone, S. Come, R. Bast, L. Schnipper, & E. Frei III, Dana Farber Cancer Institute and Beth Israel Hospital, Boston, MA.** 37 patients were treated on a phase II evaluation of a preparative regimen of cyclophosphamide (CPA), cisplatin (DDP) & BCNU. The median time to PMN >500 & platelets >20,000/ul was 19 & 22 days. There were 6 toxic deaths, 3 of infection, 2 of VOD & 2 with bleeding (1 bleeding & VOD). Transient hypertension & excessive platelet consumption (>100 units/course) occurred in 80% & 77% of patients respectively. All patients had failed prior chemotherapy save for 2 patients with breast cancer and all melanoma patients. Responses are shown by disease. 5 patients with a PR were rendered disease free by subsequent surgery or radiation. The median time to progession and survival is 6 & 20 months respectively (range 1-29+ months).

	DAY FROM MARROW REINFUSION								
	-8	-7	-6	-5	-4	-3	-2	-1	0
BM Harvest	X								
Total Dose/sqM									
CPA 5625		-	-	-					
DDP 165		----------							
BCNU 600					-				
BM Reinfusion									X

Disease	#	UE	NR	PR	CR	RR/Eval
Breast	12	1	1	6	4	91%
Melanoma	12	1	6	5	0	45%
Sarcoma	6	2	0	4	0	100%
Lymphoma	3	0	0	2	1	3/3
Ovarian	2	0	0	2	0	2/2
Other	2	1	0	1	0	1/1
Total	**37**	**5**	**7**	**20**	**5**	**78%**

UE Unevaluable NR No Response

P137 THE USE OF HIGH DOSE CYCLOPHOSPHAMATE BCNU AND VP-16 (CBV) AND AUTOLOGOUS OR ALLOGENEIC BONE MARROW TRANSPLANTATION (AUTO-BMT, ALLO-BMT) IN THE TREATMENT OF ACUTE LEUKEMIA (AL); Karel A. Dicke, M.D. Anderson Hospital and Tumor Institute.

The high dose CBV program (cytoxan 6.0/m^2, BCNU 300mg/m^2, and VP-16 750mg/m^2) has been used in the treatment of acute leukemia (AL). We treated 29 AL patients (pts) in relapse (16 ALL, 13 AML pts) with CBV and auto-transplants. Except in 1 pt, CBV was used as 2nd or subsequent salvage therapy. CR after CBV as 2nd salvage was 58% (7 of 12 pts) and as 3rd salvage 42% (5 of 12 pts). Thirteen pts were treated in 2nd relapse, CR rate 54% (7 of 13 pts) and 5 pts in 3rd relapse, CR rate 40% (2 of 5 pts). Only 11 pts were treated in 1st relapse of which 5 (45%) achieved CR. The median remission duration is 3.5 months (1-8). Five ALL pts were treated with CBV in CR2 of which 1 pt is still alive, 2 pts treated in CR3 relapsed 5 mos after BMT. In AML, 10 pts were treated in CR2, 2 are still in CR, 5+ and 36+ mos. Due to myelosuppression 8 pts relapsed. Two pts were treated in CR3, relapse occurred 3 and 12 mos after BMT. In CR1, 7 AML pts of which 1 relapsed were treated, follow-up 6 mos (1-12), 6 ALL pts of which nobody relapsed, follow-up 7 mos (4-15). These results are too preliminary for definite evaluation. The anti-leukemic effect of CBV was studied in the allogeneic setting. Three of the 4 pts treated with CBV and allo-transplants from HLA-identical MLC negative related donors in CR2 are still alive, 38+, 12+, 2+ mos; whereas 7 of 7 pts died after CBV, transplanted in CR3. Death was due to GVH (4) and relapse (3). These data are not different from the results of allo-transplantation after piperazinedione and total body irradiation (TBI).

P138 USE OF CHLORHEXIDINE TO REDUCE ORAL DISEASES AND ASSOCIATED SYSTEMIC COMPLICATIONS IN BONE MARROW TRANSPLANT PATIENTS. G. Ferretti*, R. Ash, A. Brown, J. Henslee, J. Macdonald and T. Lillich. U. of KY, Lex. KY.

The oral and systemic effects of chlorhexidine were evaluated in a 90 day double-blind study of 56 bone marrow transplant patients assigned to either an experimental group that received a 0.12% chlorhexidine gluconate mouthrinse three times daily or to a placebo group. Oral streptococci, candida, mucositis, gingivitis and plaque were scored using standard methods. Data on morphine use and on microorganisms isolated from sites other than the mouth were obtained from patient records. There were significant reductions of oral streptococci, yeast, plaque, gingivitis, and mucositis in the chlorhexidine group. Morphine use related to oral pain was higher in control patients. However, there were no differences between the two groups in the frequency with which microorganisms were isolated from blood, urine, stool, and throat samples. It can be concluded that chlorhexidine significantly reduces the oral infectious challenge of endogenous streptococci and yeast, and the incidence and severity of oral mucositis, gingivitis and plaque. Data from this study also suggest that this oral antimicrobial agent reduces morbidity as measured by the decreased use of IV morphine in the group receiving chlorhexidine. (This study was supported by the Univ. of KY and by a grant from the Procter and Gamble Company, Inc.).

P139 USE OF MAGNETIC MICROSPHERES & MONOCLONAL ANTIBODIES FOR PURGING BONE MARROW FOR USE IN AUTOLOGOUS & HAPLOTYPE-MISMATCHED TRANSPLANTATION, Adrian Gee, Douglas Barrett, Carlos Lee, Karen Bruce, William Janssen, John Ugelstad*, John Kemshead** & Samuel Gross, Univ. of Florida, Gainesville, FL 32610, *Univ. of Trondheim, Norway, **Imperial Cancer Research Fund, London, England.

The magnetic system, developed by Kemshead et al for purging neuroblastoma, has been modified and used to treat 9 patients at the Univ. of FL. Marrows from 2 other institutions have been flown to FL for purging, and returned in liquid N_2 for reinfusion at the referring hospital. 5/5 patients have engrafted and 4/5 remain disease-free at periods up to 5 months post-transplant. The procedure has been adapted for the removal of cALLa-positive leukemia cells, using three monoclonal antibodies (MoAbs): J5, AL-2 and OU-ALL-1, directed against the cALLa antigen & a cALLa-associated antigen. Experiments in which cALLa-positive leukemia cells were pre-labeled with the Hoechst fluorochrome Bisbenzimide H33342 & seeded into simulated bone marrow, indicate that more than 99.9% can be removed by a single treatment. The magnetic system has also been adapted for the removal of T cells from normal bone marrow using MoAbs to the T3 (CD3) and T11 (CD2) T cell surface antigens. Greater than 99% removal of T cells can be achieved with retention of colony forming activity by the treated marrow. T cell functional activity, as measured by MLC, blastogenesis assays and IL-2-dependent outgrowth, showed a parallel decrease after purging. These results indicate that depletion of subpopulations of cells from bone marrow can be achieved with high efficiency using MoAbs & magnetic microspheres & that this procedure does not affect the ability of the purged marrow to engraft. Supported by: The Pardee Foundation & The American Cancer Society.

P140 RAPID DIAGNOSIS AND TREATMENT OF CYTOMEGALOVIRUS PNEUMONITIS, P.D. Griffiths, P.R. Stirk, H.A. Blacklock*, R.M. du Bois and H.G. Prentice, Royal Free Hospital, Great Ormond Street Hospital for Sick Children*, London, U.K.

Cytomegalovirus (CMV) replicates slowly in cell cultures with cytopathic effect (cpe) appearing a mean of 17 days post-inoculation. To provide a more rapid diagnostic method we evaluated the ability of Mabs directed against CMV "early" antigens to identify virus-infected cells before cpe became apparent. Studies revealed that when specimens of urine, saliva or blood were inoculated these antigens became detectable by immunofluorescence at 18 h post-infection. This technique is called DEAFF (detection of early antigen fluorescent foci) and has become the routine method used in this laboratory.

When bronchial lavage fluid (BLF) is inoculated, DEAFF can reveal CMV infection of the lung. CMV is not found in BLF from most seropositive immunosuppressed patients nor in those with CMV excretion from other sites including saliva. We believe therefore that this test is diagnostic of CMV pneumonitis (CMV.PN) in BMT recipients and have used it to enter 20 consecutive episodes in 17 patients into an open trial of CMV hyperimmune immunoglobulin. The immunoglobulin, prepared by BIOTEST, has a CMV titre by RIA of 1,250,000 and was given i.v. at 400mg/kg on day 0, 4, 8 with 200mg/kg at days 12 and 15. At the time of writing, 8 patients have died from CMV/PN, 2 have recovered to die later from other causes, 4 have recovered and are alive and well up to 18 mo post-diagnosis, 3 have recovered but returned 2 mo later with CMV.PN which responded again to immunoglobulin. From this open study it appears that the immunoglobulin may be effective if given early in the course of CMV.PN.

P141 PROPHYLACTIC CMV IMMUNE GLOBULIN FOR THE PREVENTION OF CMV ASSOCIATED PNEUMONIA, Mine Harada, Takao Mori, Hisashi Funada, Tamotsu Matsuda and Kanazawa University BMT Team, Kanazawa 920, JAPAN

For the prevention of CMV associated pneumonia, CMV immune globulin was given prophylactically to 10 patients who received allogeneic bone marrow transplantation from HLA-identical siblings. All patients were maintained in a laminar air-flow room with total intestinal decontamination. Lots of relatively high anti-CMV antibody titers were selected from commercially available immune globulin. Prophylactic CMV immune globulin was intravenously infected weekly at a dose of 200 mg/kg of the recipient's body weight from day 30 to day 120 posttransplant. Serum levels of anti-CMV antibody were monitored before and during the prophylaxis. Serum levels of anti-CMV antibody increased as CMV immune globulin was given. One patient who developed systemic CMV infection did not show any increase of anti-CMV antibody titer. Despite relatively high incidence (50%) of CMV infection which was documented by the isolation of CMV and significant increase of complement fixation anti-CMV antibody titers, only one out of 10 patients developed CMV pneumonia. In contrast, 14 of 24 patients who did not receive prophylactic CMV immune globulin developed CMV pneumonia. Although these two groups of transplant patients are different in risk factors and not comparable, these preliminary results indicate that prophylactic immune globulin is effective for reducing the incidence of CMV associated pneumonia in allogeneic bone marrow transplant patients.

P142 HIGH-DOSE N,N',N''-TRIETHYLENETHIOPHOSPHORAMIDE (THIO-TEPA) AND AUTOLOGOUS MARROW TRANSPLANTATION (AMT) FOR METASTATIC MALIGNANT MELANOMA (MMM). G. Herzig, R. Brown, J. Fay, S. Wolff, K. Krupp, R. Herzig. Washington University, St. Louis, MO 63110. As part of a phase I study, 22 patients (pts), median (range) age 42 (21-70) yr, with MMM received 180-1125 mg/m² thio-TEPA and AMT. Four pts had MMM limited to skin and/or lymph nodes; 18 pts had visceral organ involvement (median [range] of 3 [1-6] organs). Although the dose-limiting toxicity in the phase I study was severe oral mucositis at the 1125 mg/m² level, no pt with MMM experienced severe mucositis and there were no fatal extramedullary toxicities. Recovery of PMN >500/ul and platelets >20K/ul was observed a median (range) of 16 (11-24) d and 18 (7-27) d after AMT, respectively. Two pts (9%) died of sepsis before recovering peripheral counts without evidence of tumor progression and were therefore not evaluable for tumor response. Of 20 evaluable pts, 9 responded, with 2 complete and 7 partial responses (rate of response >30%, alpha <0.5, beta <0.05). Of 4 pts with skin +/- lymph node involvement, 3 responded (1 CR, 2 PR); of 16 pts with extensive visceral disease, 6 responded (1 CR, 5 PR). The median (range) duration of unmaintained response was 3 (1-6+) mo; 4 of the 9 responding pts are still alive: 1+, 4+, 5+, 6+ mo. Conclusions: (1) High-dose thio-TEPA and AMT can be given with acceptable toxicity and is probably an effective regimen for pts with MMM. (2) Although the follow-up has been limited, additional maneuvers will be necessary to improve the duration of response.

P143 A PHASE I-II STUDY OF HIGH-DOSE N,N',N''-TRIETHYLENETHIOPHOSPHORAMIDE (THIO-TEPA) AND AUTOLOGOUS MARROW TRANSPLANTATION (AMT) FOR REFRACTORY MALIGNANCIES. R. Herzig, R. Brown, J. Fay, S. Wolff, M. Egorin, S. Strandjord, K. Krupp, G. Herzig, Cleveland Clinic Foundation, Cleveland, OH 44106.
In a phase I-II study, 69 patients (pts), median (range) age 48 (2-70) yr, received 180-1125 mg/m² thio-TEPA with AMT. Dose-limiting toxicity was severe oral mucositis at the 1125 mg/m² level (8/19 pts v. 0/50, $p<0.05$). There were no fatal extramedullary toxicities. A skin toxicity not previously reported with thio-TEPA- erythema &/or bronzing- was seen in 15 (22%) pts. Marrow recovery (PMN >500/ul, platelets >20K/ul) was observed a median (range) of 14 (3-42) d after AMT; only 6 pts had recovery >1 mo post-AMT. Pharmacokinetic studies of high-dose thio-TEPA were similar to conventional dose ($t_{1/2}$, clearance, distribution); the peak level and area under the curve was proportionately higher than the conventional dose. Complete and partial responses occurred in 25/59 (42%) evaluable pts. A dose-response effect was seen comparing the responses at the highest levels (5/9 & 10/16 at 900 & 1125 mg/m²) with the lowest level (0/7 at 180 mg/m²), $p<0.03$ & <0.01, respectively. Responses were seen in pts with melanoma (9/20), colon (4/9), sarcoma (1/6), unknown primary (3/4), breast (2/4), neuroblastoma (1/3), ovarian (1/3), lung (1/2), lymphoma (1/1), gastric (1/1), and Wilm's (1/1). The median (range) duration of unmaintained response was 3 (1-8+) mo. Conclusions: (1) AMT permits a significant escalation in thio-TEPA before encountering skin toxicity and dose-limiting oral mucositis; (2) pharmacokinetics of high-dose thio-TEPA parallel conventional doses; (3) a 42% response rate with a dose-response effect was observed.

P144 INTENSIVE THERAPY AND AUTOLOGOUS BONE MARROW SUPPORT (ABMS) FOR THE TREATMENT OF REFRACTORY NON-HODGKIN'S LYMPHOMA (NHL), DD Hurd, TW LeBien, BA Peterson, NKC Ramsay, EG Levine, LC Lasky, T Kim, CD Bloomfield, L Filipovich, D Vallera, PB McGlave, and JH Kersey, University of Minnesota, Minneapolis, MN 55455

Since March, 1983, 16 patients (pts) have undergone intensive chemoradiotherapy or chemotherapy alone with ABMS for the treatment of refractory NHL. 8 pts had never achieved remission (CR), 5 were in relapse after prior CR; 3 were in 2nd CR. 12 pts had their marrow treated in vitro with monoclonal antibodies BA-1 (anti-CD24), BA-2 (anti-CD9), BA-3 (anti-CD10) plus complement, 2 with T-ricin immunoconjugates, and 2 pts had no marrow treatment. The median cell dose of treated marrow infused was .58 x 10^8 nc/kg (range .23-.89); 2 pts received 1.24 and 1.49 x 10^8 nc/kg of untreated marrow, respectively. 3 pts are too early to evaluate. Time to engraftment (WBC >1000 x 3 days) occurred at a median of 23 days (range 15-59) (no difference between the 3 marrow groups). Last RBC and platelet transfusions were at a median of 24 and 31 days except for 1 pt who is transfusion dependent at day 399. 3 pts died following ABMS at day 10, 14, and 43 (2 with disease), 3 others failed to clear their NHL (2 died at day 61 and 119, 1 alive at day 186); 1 pt died at day 140 of interstitial pneumonitis with no evidence of NHL. No pt has relapsed who had a documented CR following this therapy. The Kaplan-Meier projection at 32 months for disease free survival is 55% ± 15 (standard error) and for overall survival is 52% ± 14. Our data suggest that intensive therapy with ABMS is effective for refractory NHL, and in vitro marrow treatment does not adversely influence engraftment. Further improvements are needed in the preparative regimen to eradicate resistant lymphoma.

P145 AUTOLOGOUS BONE MARROW TRANSPLANT (ABMT) AFTER FRACTIONATED TBI (200 cGy x 6) AND HDARA-C (3 g/m^2 x 8) IN TWO PATIENTS WITH RESISTANT T CELL LYMPHOBLASTIC LYMPHOMA.

Ferremi P., Morrica B., Rossi G., Belletti S., Roncoli B., Verzura P., Franceschini F., Moretti L., Izzi T., Magno L. and Marinone G.
Spedali Civili di Brescia - Ospedale di Pesaro. Italy.

Two patients (P.F. and B.A.) affected by T cell lymphoblastic lymphoma resistant to conventional treatment were transplanted with autologous cryopreserved bone marrow after FTBI and HDARA-C. They received TBI in 6 fractionated doses of 200 cGY x 6 by a 22 MV linear accelerator and HDARA-C 3 g/m^2 for 8 doses. The cell dose transplanted was 0.5 and 0.6 x 10^8/Kg. The cryopreservation was performed one month before the reinfusion and the patients were followed in air filtered rooms with reverse protective isolation. P.F. a 24 years old patient, presented a mediastinal and meningeal relapse during treatment with CHOP. He was transplanted in July 16, left isolation at +21 and was free of disease at dimission at +31. The patient is currently alive and well. B.A. a 19 years old patient was affected by a resistant bilateral neck and mediastinal lymphoma. He was transplanted in August 28. At day +5 he presented a severe progressive VOD (hepatomegaly, ascite, azotemia, encephalopaty) and died at +10.

P146 EVALUATION OF AUTOLOGOUS MARROW USING IN VITRO COMMITTED AND MULTIPOTENT PROGENITOR GROWTH, L.C. Lasky, E.D. Zanjani, J. McCullough, N.K.C. Ramsay, D.D. Hurd, T. LeBien, J. Kersey, University of Minnesota, Minneapolis, Minnesota 55455

A method for in vitro evaluation of marrow infusate's ability to promote hematopoietic recovery before autologous use (ABMT) is desirable, especially when the marrow has been treated to remove tumor cells. We have examined the committed and multipotent hematopoietic progenitor content of infused marrow in 26 ABMT patients with acute lymphoblastic leukemia in remission (22) or non-Hodgkin's lymphoma(4) (Group 1). Remission marrow was treated prior to cryopreservation with monoclonal antibodies BA-1,BA-2,BA-3 (anti-CD24,-CD9, and -CD10, respectively) and complement to remove residual tumor cells. We also studied 19 ABMT patients with solid tumors (17), CML (1), and lymphoma (1) in which marrow was frozen without purging (Group 2). Cultures for multipotent CFU-MIX and the committed erythroid (BFU-E and CFU-E) and myeloid (CFU-GM) progenitors were performed in up to 3 different culture systems: plasma clot, methylcellulose, and agar. All patients engrafted. The marrow from each patient grew in at least one system. Since culture may fail for a variety of reasons besides progenitor absence, values for progenitors that did not grow were considered missing in subsequent analysis. In group 1, the number of days until WBC exceeded 1000/µl correlated with the numbers of CFU-MIX (r=-.46,p=.007, Spearman) and BFU-E (r=-.45,p=.012) infused. CFU-GM and BFU-E were related to the day on which the neutrophil count exceeded 500/µl (r=-.56,p=.029 and r=-.41,p=.023). BFU-E helped predict the red cell transfusion requirement during weeks 7 and 8 (r=-.50,p=.007); low numbers of BFU-E infused were related to a prolonged post-ABMT red cell transfusion requirement (Fisher's exact p=.017). In group 2, CFU-MIX and CFU-GM correlated somewhat with days until WBC > 1000/µl (r=-.52,p=.03 and r=-.5,p=.04). CFU-MIX level showed an inverse relationship to days to neutrophil count > 500/µl (Fisher's exact p=.02). The CFU-GM correlated with day 28 reticulocyte count, when the latter was available (r=.79,p=.01,n=8). This study demonstrates a low but significant contribution of the various assayable progenitors to post-ABMT recovery. A battery of progenitor cultures may be helpful in evaluating marrow for ABMT.

P147 IMMUNOLOGICAL RECONSTITUTION AND CARDIAC ALLOGRAFT SURVIVAL IN RHESUS MONKEYS CONDITIONED WITH IRRADIATION AND T CELL DEPLETED AUTOLOGOUS MARROW TRANSPLANTATION,

R. D. Moses, R. E. Clark, P. J. Lucas, R. R. Quinones, D. H. Sachs, and R. E. Gress, Surgery Branch, NHLBI, and Immunology Branch, NCI, Bethesda, MD 20892

Immunological reconstitution and cardiac allograft survival have been studied in rhesus monkeys prepared by total body irradiation and T cell depleted autologous bone marrow transplantation (BMT). Individual experiments paired a treatment (Rx) with a control (Cn) animal. Rx marrow was depleted of T cells by E-rosetting and anti-human T cell monoclonal antibodies plus complement to $\leq$ 0.008% residual T cells by limiting dilution assay. Cn marrow was not depleted and contained $\geq$ 3.8% T cells. By day 40 after transplantation, total peripheral lymphocytes, 2H7+ B cells, and Leu 2+ suppressor/cytotoxic T cells had reconstituted to about 50% pre-BMT values in the Rx and Cn. In both, Leu 3+ helper T cells were profoundly depressed at this time, and showed minimal return through day 160. Mitogen induced proliferation and MLR remained depressed for Rx through day 160 while CML returned to pre-BMT levels by day 124. Rx CML was restored at day 54 by added TCGF. Functional reconstitution in Cn was similar except for better mitogen responses. Longest heart survival was 160 days in Rx and 24 days in Cn. We conclude that the pattern of immunological reconstitution in the extensively T cell depleted autologous marrow-transplanted animals is not strikingly different from that of the control recipients, that heart allograft rejection is delayed by marrow T cell depletion despite this similar reconstitution, and that a primary immunological defect in both groups is a persistent depression of Leu 3+ cell numbers and T helper function.

P148 IMMUNOMAGNETIC MARROW PURGING PROCEDURE IN BURKITT'S LYMPHOMA-QUANTIFICATION BY A LIQUID CELL CULTURE ASSAY. M.C. Favrot, I. Philip and T. Philip, Centre Leon Berard, 28 rue Laennec, 69373 Lyon Cedex 08, France.

An immunomagnetic depletion technique has been described in neuroblastoma for removing malignant cells from bone marrow (BM) prior to autologous transplantation. The development of a liquid cell culture assay that detects up to a 5 log elimination of Burkitt's lymphoma cells from 1% contaminated human bone marrow has enabled the evaluation of this procedure for Burkitt's lymphoma. The initial technique was modified by a better coating of magnetic particles with sheep anti-mouse immunoglobulin, ficoll separation of BM mononuclear cells, the use of a higher proportion of beads and, finally, by a second incubation with beads, followed by a second magnetic separation.

Under these conditions, the immunomagnetic procedure is able to remove more than 5 log BL cells from 1% contaminated samples. Results were obtained either on EBV+ african cell lines or EBV(-) caucasian cell lines freshly established from patients' tumors. The modifications in the procedure did not appear to significantly increase its toxicity on progenitor cells. In a series of comparative experiments, the efficiency of this technique and of the complement lysis procedure, using the same monoclonal antibodies, were very similar. Furthermore, there is evidence that some lines resistant to these two procedures may be purged effectively with ASTA.Z 7557.

P149 CMV PNEUMONITIS IN RECIPIENTS OF MATCHED T-CELL DEPLETED ALLOGENEIC BMT.

Grob, J-P., Grundy, J.E., Prentice, H.G., Griffiths, P., Brenner, M.K., Milburn, H.J., duBois, R., Skeggs, D., Hoffbrand, A.V. Royal Free Hospital, London, U.K.

From 2/83 to 11/85, 48 patients with haematologic malignancies (22 AML, 14 AML, 12 CGL) at various stages of their disease (20 CR1, 7 CR2, 1 CR3, 8 REL-REFR, 8CP1, 2 ACCELL, 2 BT) have received BMT from HLA-matched (n = 45) or partially mismatched (n = 3) sibling donors and were at risk for CMV pneumonitis (>90d). Conditioning of BMT consisted of Cy 60mg/kg x 2 and TBI (n = 37) or Ara-C 3g/m² x 6, Cy 45mg/kg x 2 and TBI (n = 11). TBI was given in a single fraction at 26cGy/min in air using an 8MeV LA at a prescribed dose of 7.5Gy (n = 43) or recently 8Gy (n = 5). Donor marrow was depleted of T lymphocytes. Eight patients (6F, 2M; age range 16-41;5 AML, 2CGL, 1 ALL) developed CMV pneumonitis, diagnosed early with bronchial lavage and the detection of the appearance of CMV-encoded early antigens. All were treated with CMV-hyperimmune globulin, 5 are alive 1 to 9 months later, 2 after a second episode of CMV pneumonitis. 7 of 8 were sero.+ve prior to BMT, 7 of 8 had GvHD and had received a significantly higher lung dose of TBI than patients without CMV pneumonitis (789 ± 38cGy, median 783, range 762-843 versus 717 ± 122cGy, median 740, range 639-789). In CMV sero.+ve patients (n = 25), 7/9 patients who received >=750cGy to the lungs developed CMV pneumonitis, while 0/16 patients who received lower lung doses developed pneumonitis (p <0.001). In contrast only 2/11 seronegative recipients who received >750cGy to the lung developed pneumonitis, one due to CMV.

P150 Utility of Bronchoscopy and Bronchoalveolar Lavage (BAL) Prior to High Dose Therapy and Autologous Marrow Transplantation (AMT). SI Rennard, J Linder, MA Ghafouri, A Kessinger, JO Armitage, MA Arneson and WP Vaughan. Departments of Internal Medicine and Pathology, University of Nebraska Medical Center, Omaha, NE.

Pulmonary infection and respiratory failure are major causes of morbidity and death in patients (pts) undergoing high dose therapy and AMT. During a 9-month period we performed surveillance bronchoscopy and BAL on 36 pts prior to receiving such therapy. For BAL five 20 ml aliquots of sterile saline were infused and immediately withdrawn through a wedged flexible bronchoscope. The first aliquot (bronchial) was analyzed separately from the subsequent 4 (alveolar). Complications were limited to post-bronchoscopy fever (10 pts, 28%). BAL fluid was cultured and analyzed cytologically by PAP stained filter preps and Giemsa and GMS stained cytocentrifuge preps. One or more of these studies or the bronchoscopy itself was abnormal in 25 (69%) of these pts. The cytology was clinically significantly abnormal in 24 pts (2 P. carinii, 4 budding yeast, 3 macrophage or PMN-associated bacteria, 1 tumor cells, 18 purulent bronchitis, alveolitis or both). Two pts had unsuspected upper airway obstruction and 1 pt endobronchial Hodgkin's disease. Abnormal BAL was found in 7 of 10 pts who were afebrile and had normal chest x-ray and pulmonary function tests (3 budding yeast, 1 macrophage-associated bacteria and 4 purulent bronchitis). One pt with P. carinii died of that infection but the others all were managed successfully for their pre-AMT pulmonary problems. Pretreatment bronchoscopy with BAL is a well tolerated and useful approach to diagnosis and management of pulmonary problems in this high risk population even if the chest x-ray and pulmonary function is normal.

P151 DEPLETION OF T LEUKEMIA CELLS FROM BONE MARROW USING MONOCLONAL ANTIBODIES AND MAGNETIC IMMUNOBEADS. C.P. Reynolds*, A.J. Melaragno*, A.T. Black*, D. Vembu*, J.A. Maples*, and J. Ugelstad*. Transplantation Research Program Center, Naval Medical Research Institute, Bethesda, MD, and Laboratory of Industrial Chemistry, University of Trondheim, Trondheim, Norway.

To study selective depletion of T leukemia cells (TLC) from marrow, we developed a model system in which TLC marked with the supravital DNA stain Hoechst 33342 (H342) are seeded into bone marrow (1 H342-stained cell per million marrow cells can be detected). Marrow/leukemia mixtures were incubated with monoclonal antibodies (Mabs), then with goat anti-mouse Ig-coated magnetic microspheres. Cells attached to microspheres can be removed with high-energy magnets. Cocktails of Mabs which produced the highest mean fluorescence on leukemia cells (as determined by flow cytometry) were most effective at target cell removal. From a panel of 16 anti-T cell Mabs, a cocktail of 6 Mabs which produced the brightest staining of TLC resulted in 3.4, 4.0, and 4.8 log reductions in human leukemia cells seeded into bone marrow for 3 TLC lines tested (20% TLC seeded). Little or no reduction of cell viability or myeloid colonies was seen. Immunomagnetic purging should allow use of autologous marrow transplant for T leukemia patients, and may be useful for cell depletion of allogeneic marrow. NMR&DC MF58.527.004.0004

P152 A PILOT TRIAL USING FOSCARNET FOR CYTOMEGALOVIRUS INFECTIONS IN MARROW TRANSPLANT RECIPIENTS. O. Ringdén, B. Lönnqvist, T. Paulin, P. Ljungman, B. Wahren, J-O. Lernestedt. Huddinge Hospital, Stockholm, Sweden

Foscarnet inhibits viral DNA polymerase and viral multiplication of all human herpes viruses in vitro. We treated 20 marrow transplant recipients for 23 episodes with i.v. foscarnet. The indications were interstitial pneumonitis (14 episodes), pancytopenia and fever (5), fever (2), hepatitis (1), and encephalitis (1). Cytomegalovirus (CMV) infection was identified by immunofluorescence, cultures or serology: appearance of CMV IgM antibodies (>50), CMV seroconversion (>50), a 5-fold increase of IgG titers. Foscarnet was given as a bolus of 9-20 mg/kg and thereafter as a continuous infusion of 0.078-0.14 mg/kg/min. Daily dose ranged from 75-244 mg/kg, treatment duration from 2-36 days, total dose 2-399 g, average steady state 42-184 µg/ml and maximal plasma concentration 61-447 µg/ml. Clinical improvements by irradication of CMV, afebrility, improvements of laboratory abnormalities or chest X-rays were seen in 13/20 (65%) of assessable episodes. Although chest X-rays clearly improved in 3 patients all 14 with pneumonitis died. Eight of those had other complicating illnesses such as serious infections, severe GVHD or veno-occlusive liver disease. Among the remaining 6 patients one died of GVHD and 5 improved. Patients at risk for developing CMV pneumonitis have to be identified and treated early to prevent development of respiratory failure. Foscarnet is only virostatic and treatment probably has to be continued until the patient's immunity against CMV is recovered.

P153 LONG-TERM SURVIVAL AFTER AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR ADVANCED NON-HODGKIN'S LYMPHOMA, Mikio Ueda, Mine Harada, Takashi Yoshida, Tamotsu Masuda and Kanazawa University BMT Team, Kanazawa University , Kanazawa 920, JAPAN

Seven patients with advanced non-Hodgkin's lymphoma of unfavorable histology were treated with high-dose cyclophosphamide(CY) and 10 Gy total body irradiation(TBI) followed by autologous bone marrow transplantation(auto-BMT). All patients achieved complete remission(CR). Three of these patients are now surviving 56-67 mo after auto-BMT; disease-free survival in 2 and maintained CR in 1. Recurrence of the disease was observed in 3 patients at 2, 13 and 14.5 mo after auto-BMT. One of them responded to conventional chemotherapy for remission induction and is now alive in CR. Primary causes of death were relapse in 2 and interstitial pneumonia of unknown eitology in 2. Treatment failure was evaluated in 5 patients who died or relapsed after auto-BMT. Of 3 patients who developed relapse, 2 received auto-BMT during partial response in response to prior therapy. Contamination of cryopreserved marrow with tumor cells was highly suspected in the remaining patient who showed a leukemoid reaction in bone marrow pictures. Two patients developed fatal interstitial pneumonia; one had had radiation pneumonitis probably caused by the prior therapy before auto-BMT. These observations suggest that auto-BMT is a treatment of chocie with acceptible risks for selected patients with advanced non-Hodgkin's lymphoma. One of the future directionsis to consider intensive therapy with auto-BMT at an earlier stage of the disease when patients are in better clinical condition and still sensitive to chemoradiotherapy.

P154 HIGH DOSE INTENSIFICATION CHEMOTHERAPY WITH CYCLOPHOSPHAMIDE, ETOPOSIDE AND CISPLATINUM WITH AUTOLOGOUS BONE MARROW SUPPORT FOR PATIENTS WITH RESPONDING TUMORS: INITIAL EXPERIENCE WITH A PHASE I-II TRIAL. G.J. Ventura, G. Spitzer, K.A. Dicke, S. Jaganath L.J. Horwitz and JA Neidhart. M.D. Anderson Hospital and Tumor Institute, Houston, TX. 77030.

Previous investigation utilizing high dose intensification therapy (cyclophosphamide (CTX) etoposide (VP-16) and vincristine) with autologous bone marrow transplantation (ABMT) in patients with small cell bronchogenic carcinoma (SCBC) has shown the capability of such a program to (a) convert a significant percentage of patients with partial remission (PR) to complete remission (CR) and (b) possibly increase the long term disease free survival of patients who had achieved a CR with initial induction therapy. Recent evidence of the efficacy of platinum containing regimens in treating a broad range of solid tumors has led us to combine this drug with high dose CTX and VP-16 with autologous bone marrow support in treating patients with solid tumors who have shown a response to prior chemotherapy. Herein we report our initial experience with 7 patients. Six patients had SCBC (5 limited stage, 1 extensive) and 1 patient had metastatic breast carcinoma to the lungs and mediastinum. Induction therapy for SCBC consisted of 3 courses of ECHO (VP-16 60mg/m2 x 3, CTX 600mg/m2 x 3, adriamycin 50mg/m2 and vincristine 1.5mg x 2). Of the 5 limited stage patients there were 3 CRs and 2 PRs with induction therapy; the one patient with extensive disease achieved a PR. The patient with metastatic breast cancer had obtained a CR with 4 courses of CTX 600mg/m2 and adriamycin 60mg/m2. High dose intensification consisted of CTX 1.5gm/m2 x 3, VP-16 250mg/m2 x 3 and cisplatin 100-150 mg/m2. Two courses of this therapy were given 3-4 weeks apart with ABMT. At this time 6 pats.. are evaluable for toxicity and response. Mean duration of neutrophil count under 1000/mm3, 500 /mm3 and 100/mm3 was 14.5, 13 and 5 days respectively for the first course and 17, 15 and 11 days for the second course. Mean duration of platelet count less than 100,000/mm3, 50,000/mm3 and 20,000/mm3 was 7,5 and 5 days respectively for the first course and 23, 15 and 18 days for the second course. FUO occured in 75% of treatments; one patient had reversible renal insufficiency. Of the 4 patients treated while in CR, all remain in documented CR with disease free survival off therapy of at least 50,46,12 and 28 weeks. One patient with extensive SCBC converted from PR to CR with a disease free survival of 50 weeks prior to relapse in the CNS (no prior cranial irradiation). One patient with PR of limited SCBC converted to possible CR (residual abnormality on CT scan, bronchoscopy negative). Although the patient number is small it appears that the toxicity is for the most part predictable and tolerable in this older (median age 51 years) group of patients. Further patient accrural is necessary to answer the question of whether this particular type of intensification therapy will significantly extend the disease free survival of patients who have achieved prior response with conventional chemotherapy.

Late Additions

P155 Early and late myeloid progenitor cells in the peripheral blood (PB) of AML-patients after induction chemotherapy

K.Geissler,W.Hinterberger,K.Lechner
1st Dept.Med,Div.Haematol,Univ.Vienna,Austria

Early (CFU-MIX) and late (CFU-GM) myeloid progenitor cells were measured in the PB of 10 AML-patients as they entered first remission.Peak levels of both colony types occured generally during the third or fourth week after the end of induction chemotherapy.The average peak levels of both progenitor cell classes were significantly elevated compared to the mean values in 15 normal subjects,representing a 24 fold increase for CFU-GM and a 6.5 fold increase for CFU-MIX.In 3 AML-patients,who did not achieve remission,progenitor cells of both types remained below the ranges of normals.Our results indicate elevated levels of circulating myeloid progenitor cells in early remission and demonstrate a preferential expansion of circulating CFU-GM above CFU-MIX.Thus one has to be extremely cautious in predicting the reconstitutive capacity of PB during haemopoetic regeneration by CFU-GM determinations only.

P156 LYMPHADENOPATHY AIDS VIRUS INFECTION AND TRANSFUSION : THEIR IMPLICATIONS IN BONE MARROW TRANSPLANTATION.

E VILMER, A DEVERGIE, E GLUCKMAN. Unité Fonctionnelle de Greffe de Moëlle Osseuse (UFGM) Service d'Hématologie du Pr M. BOIRON - HOPITAL SAINT LOUIS 1 av Claude Vellefaux 75475 PARIS CEDEX 10 FRANCE.

The clinical aspects of LAV infection, the incidence of LAV antibodies in bone marrow donors and recipients and the therapeutic methods are studied and reported. The clinical spectrum of LAV infection is detailed : the occurrence of delayed opportunistic infections, long after the graft, the various hematological features-cytopenias, pseudo-lymphoma- are described in 5 patients (pts). Pre BMT, LAV antibody screening in the series of 125 donors-recipients pairs grafted since 1982 were performed using at least 2 or 3 methods, Elisa, Western Blot, or radio-immuno-precipitation assay. Furthermore, 59 out of the 125 pts were regularly tested following graft. The high seroprevalence before (4 %) and after BMT (11,8 %) does warrant LAV antibody screening in this population. We show how these pts with severe LAV infection may be successfully treated, using lymphocyte transfusion from the BM donor and Interferon. Our latest results provide an interesting model to propose other therapeutic trials in pts with AIDS belonging to other high risk groups.